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THE PREPARATION AND STRUCTURAL ELUCIDATION OF SOME XANTHENYLSULFANILAMIDES

by

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A THESIS

Submitted to the Faculty of Graduate Studies in
partial fulfilment of the requirements for the
degree of Doctor of Philosophy.

FACULTY OF PHARMACY

September 30, 1965 EDMONTON, ALBERTA

To

my wife Vi,

whose devotion, sacrifices

and encouragement made the realization

of this manuscript considerably less arduous.

ABSTRACT

Our interest in the identification and differentiation of bacteriostatic and non-bacteriostatic (diuretic type) sulfonamides led us to an investigation of the reaction between xanthydrol and some therapeutically important sulfanilamides of the former type. The resulting products were found to be very unstable towards heat, peroxides, and possibly other factors, creating problems in obtaining analytically pure samples. The above difficulty was overcome by performing the recrystallizations from selected miscible solvent pairs, at room temperature. Elemental analyses revealed the recovery of some dixanthenyl as well as the expected monosubstituted products, regardless of whether a deficiency or an excess of xanthydrol was present. Studies on model compounds, qualitative reactions and spectroscopic characteristics were employed in the elucidation of the structures of these xanthenyl derivatives. Some experiments illustrating the exceptional reactivity of xanthydrol were performed, and a possible explanation for this great reactivity was presented. Finally, several experiments were carried out with related compounds to suggest possible further applications of this reaction.

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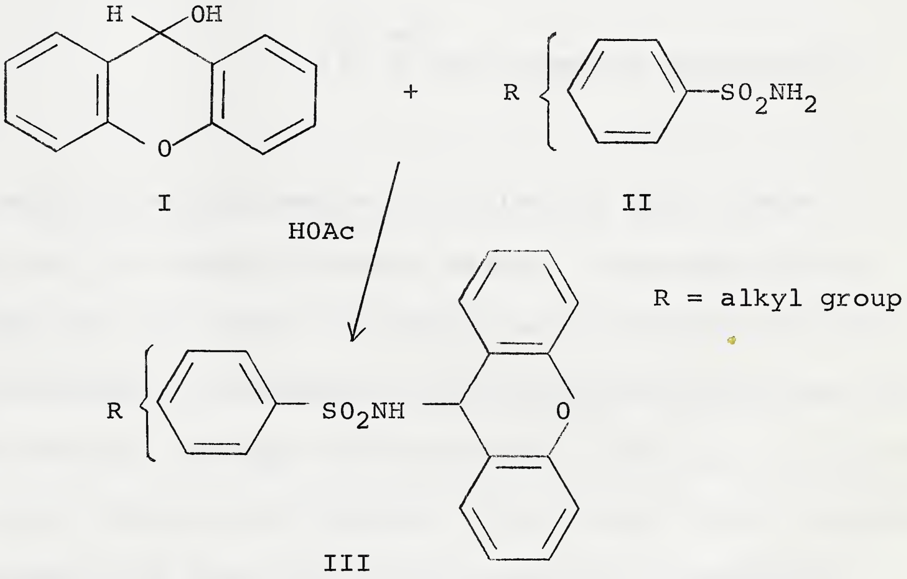
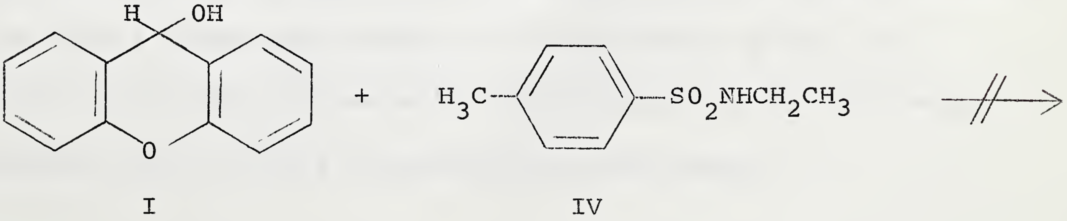
INTRODUCTION

1. The Problem

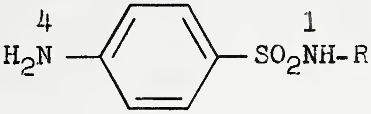
Phillips and Frank (1) in 1944 reported the reaction of xanthydrol (I) with a series of benzenoid sulfonamides (II) to yield the corresponding N-xanthenylsulfonamides (III).


H
OH
O
+
R
SO2NH2
I
II
HOAc
R = alkyl group
R
SO2NH
O
III

N-ethyl-p-toluenesulfonamide (IV) failed to condense with xanthydrol (I), confirming earlier similar observations with N-alkyl and N-aryl carboxyamides (2,3,4).


H
OH
O
+
H3C
SO2NHCH2CH3
I
IV

Since, with the exception of sulfanilamide (V), all bacteriostatic sulfonamides of therapeutic importance are N^1-monosubstituted sulfanilamides (VI), these would therefore not be expected to react with xanthydrol.

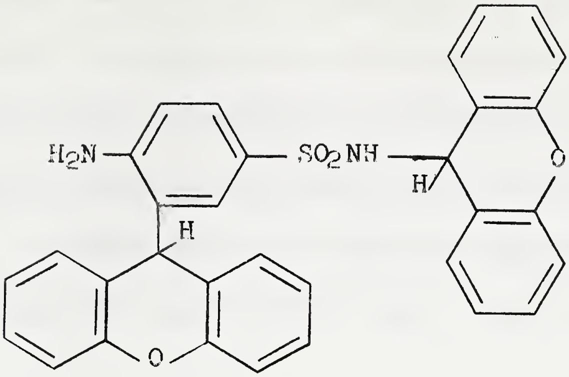


V, R=H
VI, R= Most commonly heterocyclic

However, aside from being amides of sulfanilic acid, these compounds are also primary aromatic amines. Compounds of the latter type have been shown to condense with xanthydrol to form either N-xanthenyl, C-xanthenyl or N,C-dixanthenylarylamines (3).

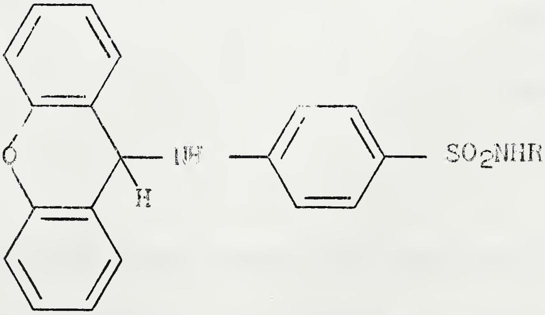
Consequently, although sulfanilamides of the type VI would not be expected to react as sulfonamides, they should behave as amines with xanthydrol and thus still afford xanthenyl derivatives.

Two reports of such a reaction are contained in the literature. Phillips and Frank (1) found that sulfanilamide (V), unlike the alkyl ring-substituted benzene sulfonamides, formed what appeared to be a dixanthenyl derivative on the basis of its nitrogen analysis. Being able to show the presence of a free amino group, by a positive test with nitrous acid, they postulated the following structure (VII) for the dixanthenyl sulfanilamide:



VII

Sawicki and Oliverio (5) condensed N^1-2-pyrimidyl (sulfadiazine) and N^1-2-pyridyl (sulfapyridine) sulfanilamide with xanthydrol, and recovered monoxanthenyl derivatives in each instance. Their infrared spectra indicated that the amino group had been substituted, and accordingly they proposed the N^4-xanthenyl structure (VIII) for these derivatives. They did not perform the nitrous acid test, nor did they make any mention of the site of substitution suggested by Phillips and Frank (1) for sulfanilamide (V).



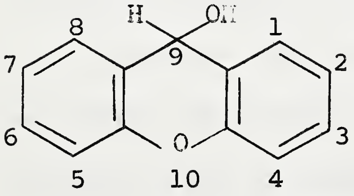
VIII. R =2-pyrimidyl or 2-pyridyl

Since a substituent in the N^1-position of sulfanilamide would hardly be expected to influence the reactivity of the *p*-amino group in this molecule, it appeared reasonable to us that a common site of substitution should have been found in each of these three sulfanilamides. As both pieces of evidence appeared equally convincing, it was felt that further work in this area was justified.

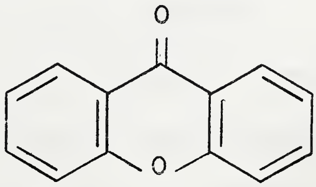
The present work was undertaken in an attempt to resolve this apparent difference in the site of substitution and also with the hope that these xanthenyl derivatives, if extended to include the whole series of bacteriostatic sulfonamides, might prove of value for their characterization.

2. Literature Survey

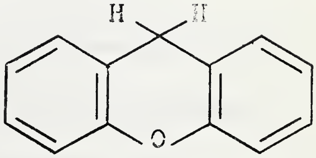
Xanthydrol (xanthen-9-ol) (I) is a heterocyclic secondary alcohol derived from xanthone (IX). The corresponding hydrocarbon is known as xanthene (X).



I



IX



X

The most generally used method for preparing xanthydrol is by the reduction of xanthone with alkaline reducing agents. Examples of such reducing agents that have been employed for this

[illegible]

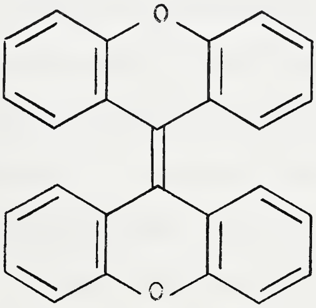
[illegible]

[illegible]

[illegible]

[illegible]

purpose include zinc and sodium hydroxide (3,6,7), aluminum and potassium hydroxide (8, 9), aluminum isopropoxide (10), and sodium amalgam and ethyl alcohol (11,12,13,14,15). The behavior with sodium and alcohol is different and gives xanthene (X)(16). Acidic reducing agents, such as zinc and hydrochloric acid, convert xanthone (IX) into dixanthylene (XI) (17).



XI

Sodium borohydride reduction of xanthone also affords xanthydrol, however, with diborane reduction does not stop at the alcohol and only the hydrocarbon (X) is recovered (18). Catalytic hydrogenation over a nickel (19) or copper (20) catalyst likewise yields mainly xanthene.

Xanthone (IX) may be conveniently prepared by the pyrolysis of phenyl salicylate (21) or by other pyrolytic or cyclization methods, many of which have been reviewed by Wawzonek (22).

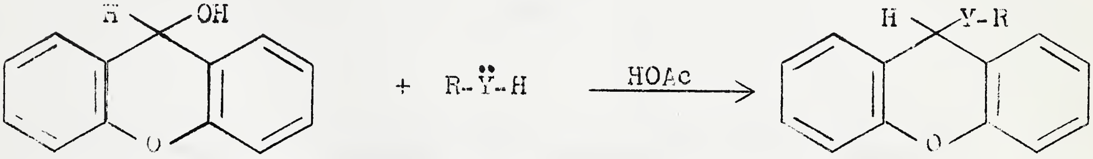
Of less practical significance, because of the relatively low yield obtained, but of theoretical interest is the direct synthesis of xanthydrol reported by Crandall, Smith and Horn (23). They obtained xanthydrol directly, in 28 per cent yield, by steam

distillation of the reaction mixture resulting from the condensation of diphenyl ether with carbon monoxide in the presence of aluminum chloride. The behavior of carbon monoxide with diphenyl ether, in bridging the ortho positions of the latter, was unique in this condensation as the other aromatic hydrocarbons studied yielded substituted anthracenes. Diphenylmethane, for example, condensed to give a dibenzylanthracene. A similar reaction of diphenyl ether with benzaldehyde to give 9-phenylxanthydrol had been observed previously (24).

Xanthydrol, because of its peculiar constitution, is an exceptionally reactive secondary alcohol. It reacts, by an electrophilic attack of its carbonium ion, with a variety of molecules containing centers of high electron density under extremely mild reaction conditions. In comparative studies, it has been shown to be more reactive than benzhydrol (25, 26, 27, 28) and in fact even more reactive than the tertiary alcohol triphenylcarbinol (27, 29). Simply mixing the reactants in glacial acetic acid at room temperature, or with slight heating, is usually sufficient to allow reaction to proceed. Comparable reactions with benzhydrol or triphenylcarbinol fail under these reaction conditions unless a condensing agent such as sulfuric acid is added.

In view of its extreme reactivity, it is not surprising then that xanthydrol has been employed in a large number of condensations with compounds containing an active hydrogen atom. Thus, xanthydrol (I) has been shown to react with many molecules con-

taining hydrogen substituted carbon, nitrogen and sulfur atoms. Reactions involving substitution of the hydroxylic hydrogen atom in xanthydrol are rare, the main reaction being of the above mentioned type involving substitution of the entire hydroxyl group yielding the corresponding 9- substituted xanthene (equation 1).



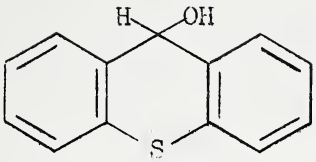
(1)

Y = C, N or S

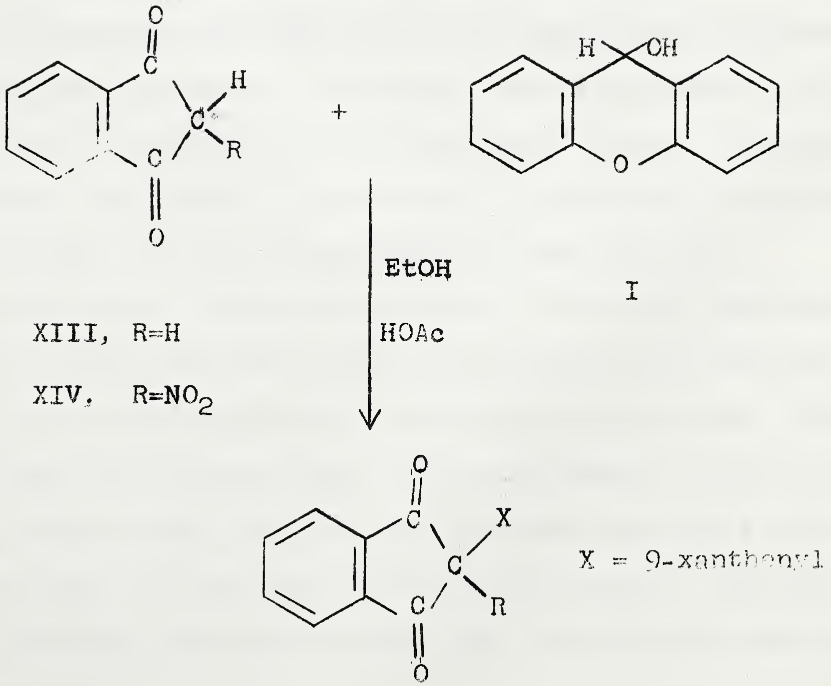
+ H_2O

Substitution at carbon occurs readily with compounds containing an active methylene group. Fosse condensed xanthydrol with aliphatic acid anhydrides (30, 31), β-keto esters, malonic ester, ethyl cyanoacetate and β-diketones (32). Sawicki and Oliverio (5) extended the series of β-diketones, and as well prepared the corresponding derivatives with thiaxanthydrol (XII). The cyclic β-diketone 1,3-indanedione (XIII) and its 2-nitro derivative (XIV) each afforded the corresponding 2-(9-xanthenyl) derivative (equation 2). Condensation of the latter compound (XIV) with xanthydrol had been reported previously by Vanags and Geita (33).

Gren and Vanags (34) studied the properties of an analog of 1.3-indanedione namely 4-cyclopentene-1, 3-dione (XV). They found that although they could not acylate or alkylate the active methylene group


XII

in XV, it readily condensed with xanthydrol (I). Thus, by simply mixing equimolar amounts of XV and I in glacial acetic acid, at room temperature, they isolated both the monoxanthenyl (XVI) and the dixanthenyl (XVII) derivatives of XV (equation 3).



XIII, R=H

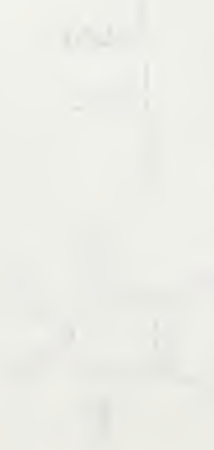
XIV, $R=NO_2$

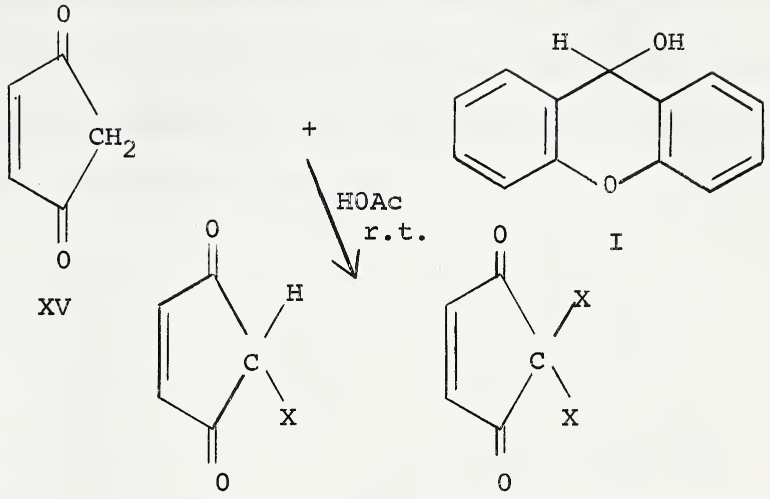
I

X = 9-xanthenyl

(2)

[illegible]



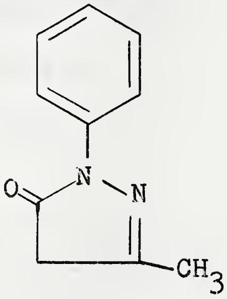


XV I XVI XVII X = 9-xanthenyl (3)

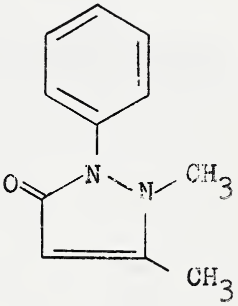
When a large excess of xanthydrol was employed in the reaction, mainly XVII was obtained. Reaction of XVI with I also yielded XVII. They also observed that the xanthenyl groups could easily be removed from XVI or XVII by the action of bromine. Compound XVII represents one of the very few examples in the literature of disubstitution by xanthenyl groups occurring on the same atom. The cyclic β-diketone 5,5-dimethyl-1,3-cyclohexanedione yielded only the monoxanthenyl compound (35).

Pastour (35), condensed xanthydrol with nine compounds containing active methylene groups by refluxing the reactants in alcohol with the addition of a few drops of pyridine. This is, to the best of our knowledge, the only example in the literature where a base rather than an acid has been employed as the condensing agent in reactions involving xanthydrol. Besides the cyclic diketone mentioned above, this procedure was employed

also for condensations with some acetoacetamides, isoxazolones and with 2-phenyl-5-methyl-3-pyrazolone (XVIII). The pyrazolone 1,5-dimethyl-2-phenyl-3-pyrazolone (antipyrine) (XIX) has also been reacted with xanthydrol, and like XVIII also yielded the 4-(9-xanthenyl) derivative (36, 37).



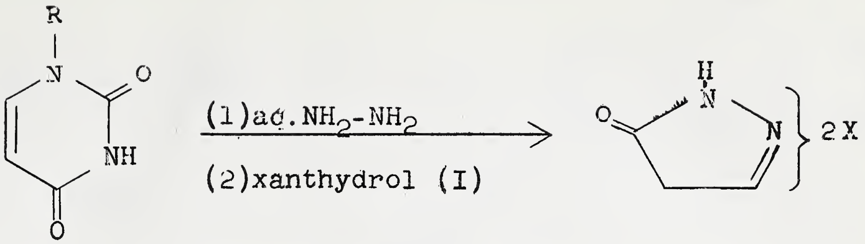
XVIII



XIX

Baron and Brown (38) reported the recovery of dixanthenyl pyrazolone (XX) during their studies on the structures of some nucleic acids. Upon carrying out a fission of uridylic acid (XXI), uridine (XXII), or 3-methyl uracil (XXIII), with aqueous hydrazine, and then treating the fission product with xanthydrol (I) they obtained dixanthenyl pyrazolone (XX). No structure was suggested, however, for this latter derivative. A similar treatment of cytidine (XXIV) gave what they assumed to be 3-amino-x, x-dixanthenyl pyrazole (XXV).

Faust and Sahyn (39), employed the reactivity of xanthydrol with active methylene groups to advantage in synthesizing a series of xanthene (and thiaxanthene) cyclic amidines. Cyano-


R
O
NH
O
(1)aq.NH$_2$-NH$_2$
(2)xanthydrol (I)
H
N
N
O
2X

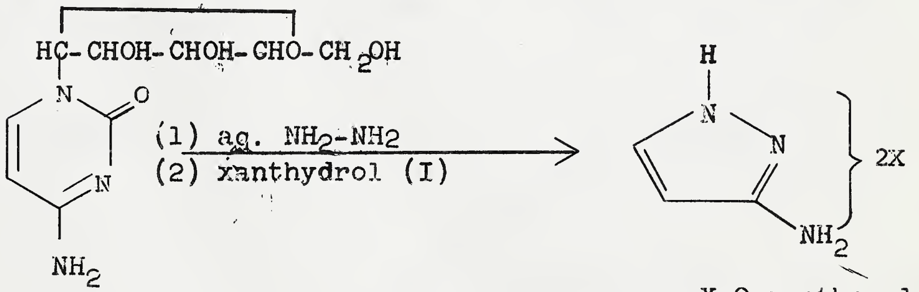
XXI, R= $-O_3H_8C_5.O.H_2O_3P$

XXII, R = $-C_5H_9O_4$

XXIII, R = $-CH_3$

XX,

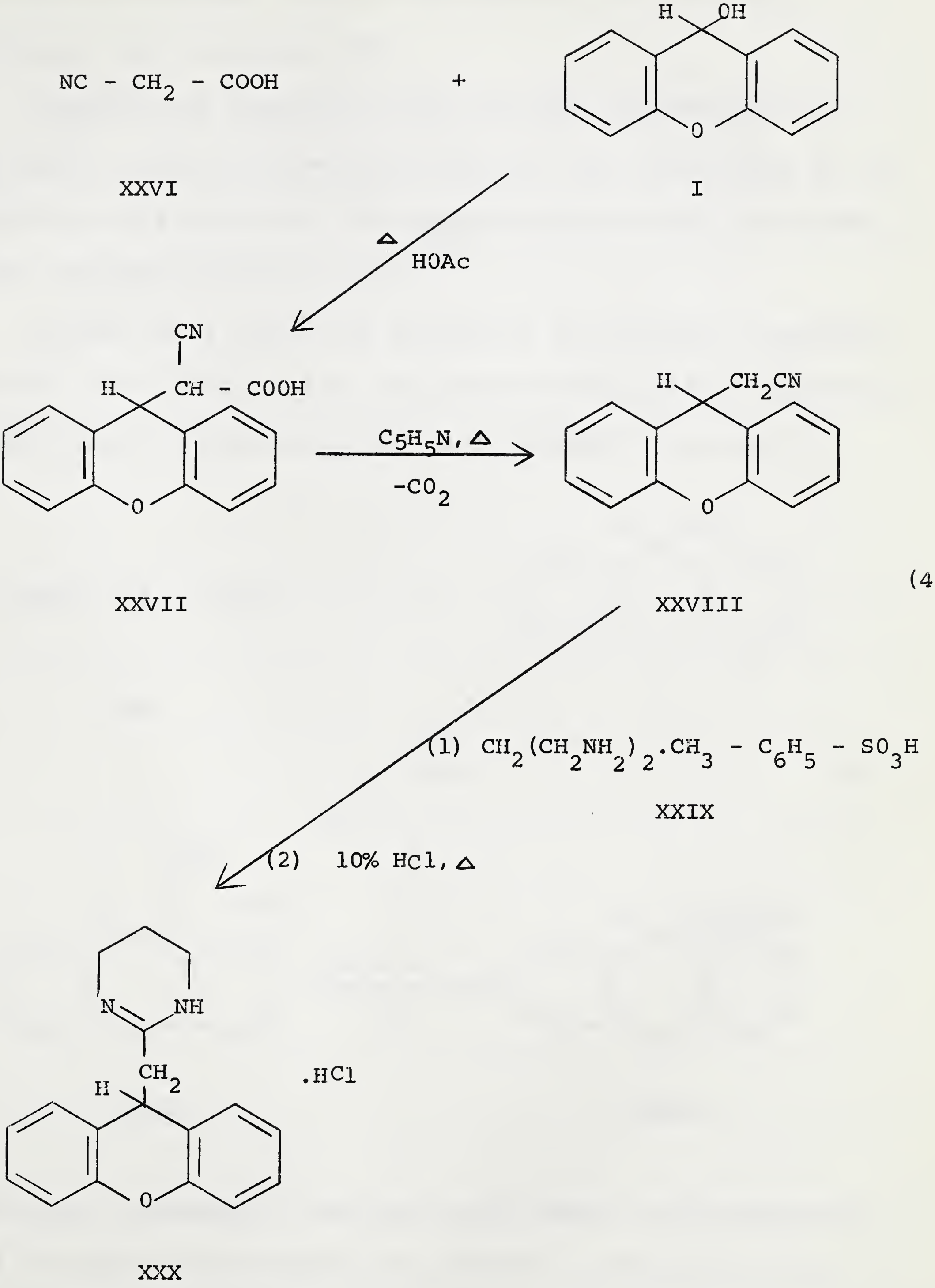
X = 9-xanthenyl


HC-CHOH-CHOH-CHO-CH$_2$OH
N
O
N
NH$_2$
(1) aq. NH$_2$-NH2
(2) xanthydrol (I)
H
N
N
NH$_2$
2X

XXIV

XXV, X=9- xanthenyl

acetic acid (XXVI) when condensed with xanthydrol (I) yielded 9-xanthenyl-cyanoacetic acid (XXVII). The latter compound (XXVII) when heated in pyridine decarboxylated to give 9-xanthenyl-acetonitrile (XXVIII). Condensation of XXVIII with a series of diamine tosylates afforded the corresponding xanthene cyclic amidines. For example, with 1,3-propanediamine monotosylate (XXIX), the cyclic amidine 9-(1,4,5,6-tetrahydro-2-pyrimidylmethyl) xanthene (XXX) was recovered (isolated as the hydrochloride salt) (equation 4).

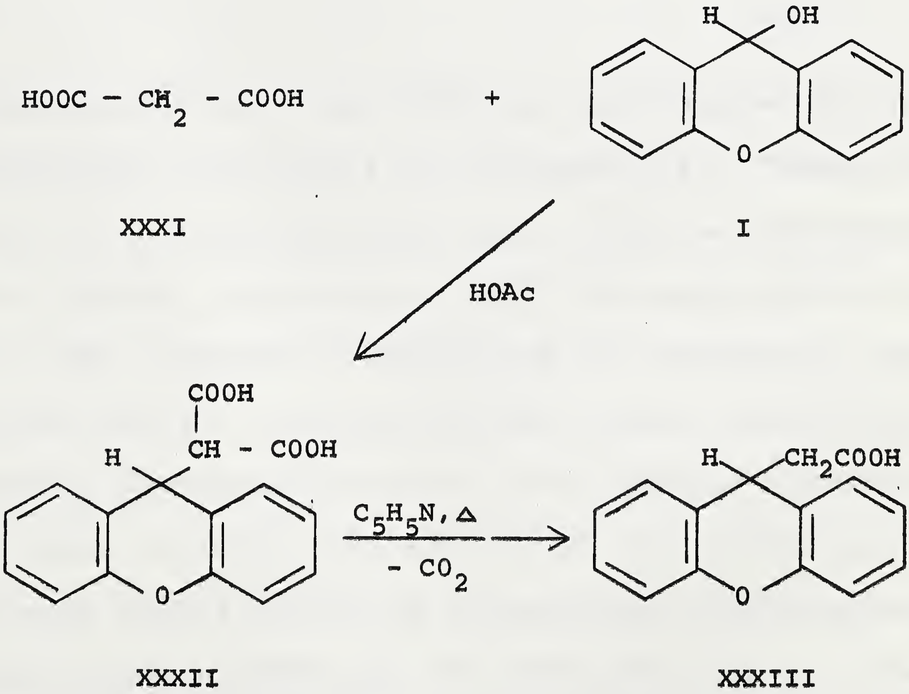

NC - CH2 - COOH
+
H OH
O
XXVI
I
Δ
HOAc
CN
H
CH - COOH
O
C5H5N, Δ
-CO2
H
CH2CN
O
XXVII
XXVIII
(1) CH2(CH2NH2)2.CH3 - C6H5 - SO3H
XXIX
(2) 10% HCl, Δ
N
NH
CH2
H
.HCl
O
XXX

(4)

The active methylene group in malononitrile has also been condensed with xanthydrol (5).

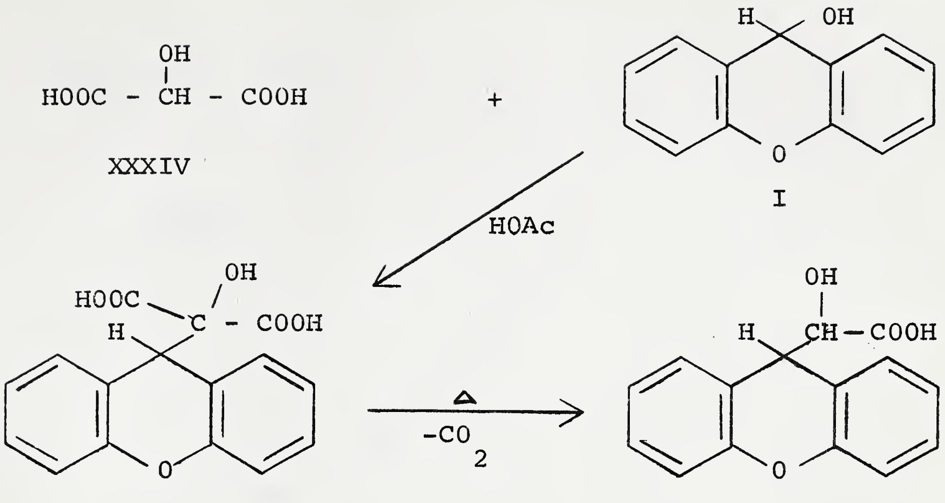
Goldberg and Wragg (15) also utilized the reactivity of xanthydrol with active methylene groups, as a first step, in the synthesis of a number of 2-dialkylaminoalkyl esters and amides of some xanthenylcarboxylic acids.

Malonic acid (XXXI) and xanthydrol (I) yielded 9-xanthenyl malonic acid (XXXII) which, when heated with pyridine, decarboxylated to give 9-xanthenylacetic acid (XXXIII) (equation 5).


HOOC – CH$_2$ - COOH
+
H
OH
O
XXXI
I
HOAc
COOH
H
CH - COOH
O
C$_5$H$_5$N, Δ
- CO$_2$
H
CH$_2$COOH
O
XXXII
XXXIII

(5)

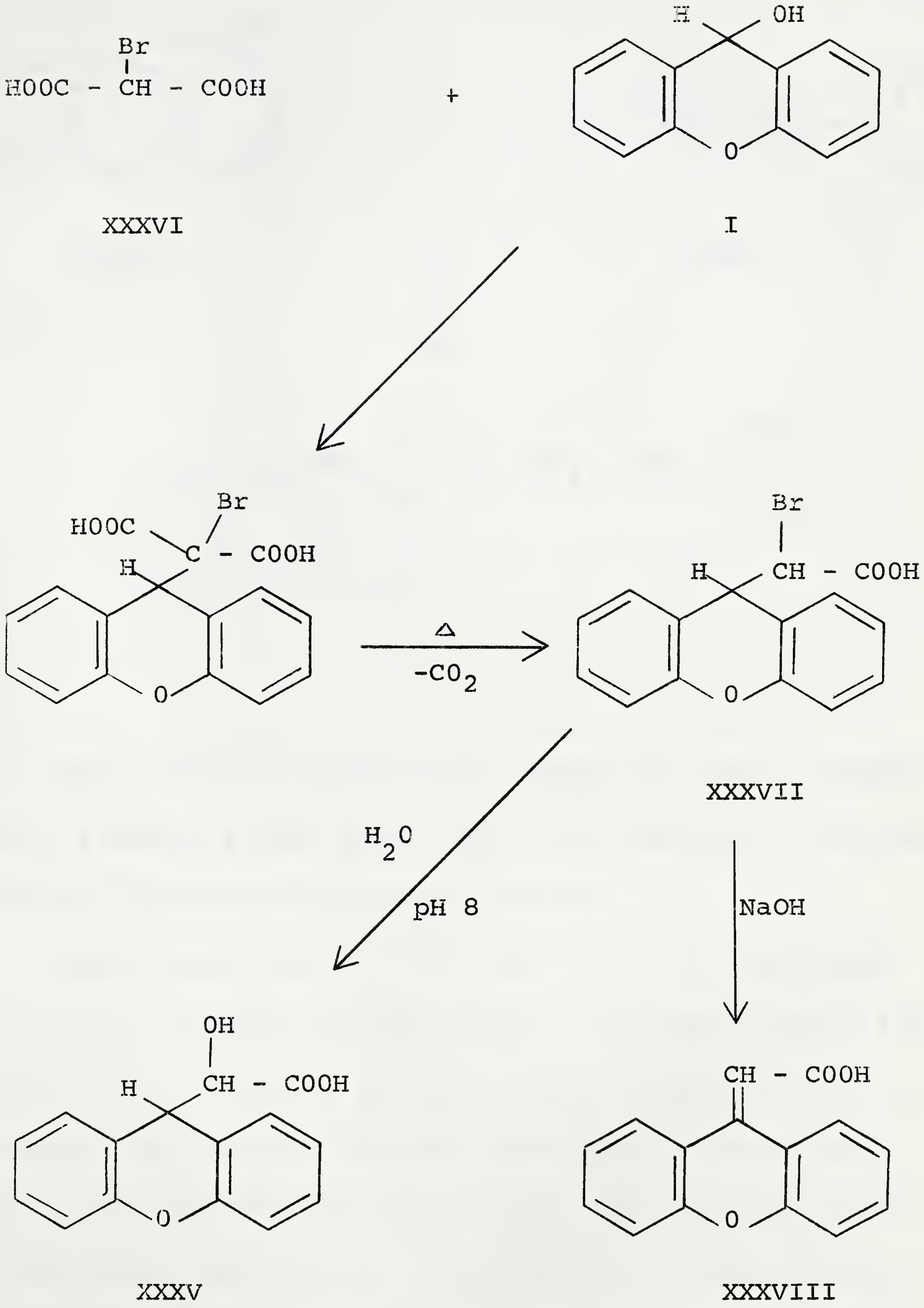
Similarly, treatment of tartronic acid (XXXIV) with xanthydrol (I) followed by decarboxylation afforded 9-xanthenylglycolic acid (XXXV) (equation 6).



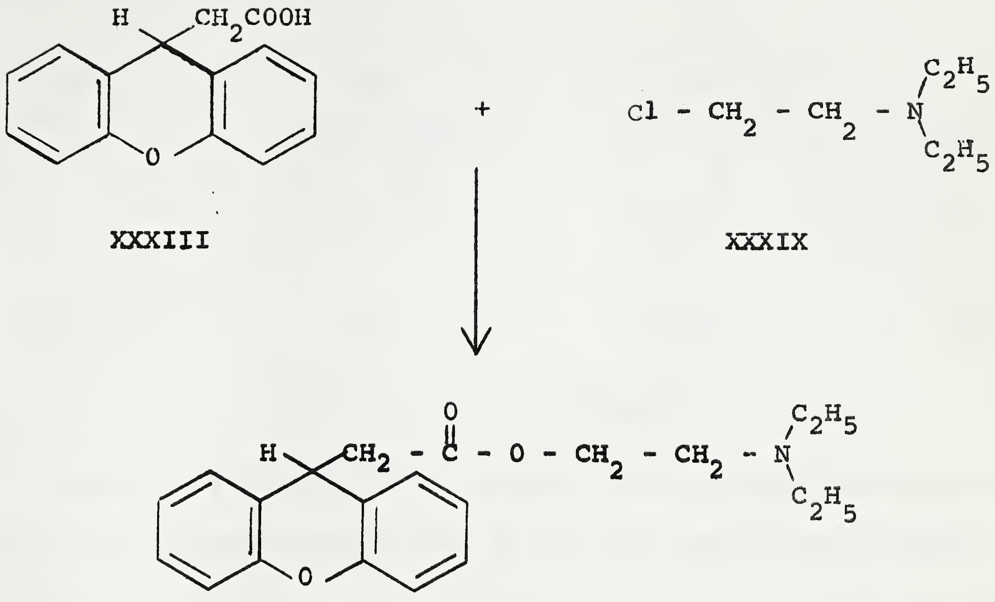
XXXV

(6)

9-Xanthenylglycolic acid (XXXV) was also prepared by condensing bromomalonic acid (XXXVI) with xanthydrol (I). Thermal decarboxylation of the resulting product gave α-bromo-α-9-xanthenylacetic acid (XXXVII). Hydrolysis of XXXVII with water buffered at pH 8 gave XXXV. Treatment of XXXVII with hot concentrated aqueous sodium hydroxide eliminated hydrogen bromide quantitatively, yielding 9-xanthenylidine-acetic acid (XXXVIII) (equation 7). Treatment of XXXIII, XXXV, and XXXVIII with 2-diethylaminoethyl chloride (XXXIX) yielded the corresponding 2-diethylaminoethyl esters (example XXXIII gave XL) which were isolated as the hydrogen oxalates or ethobromides. A similar condensation of XXXIII with 2-diethylaminoethylamine afforded the corresponding amide.


XXXVI
+
I
Δ
$-CO_2$
XXXVII
H_2O
pH 8
NaOH
XXXV
XXXVIII

(7)



XXXIII

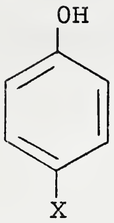
XXXIX

XL

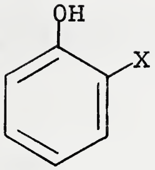
Substitution at carbon also occurs with active aromatic rings, yielding either mono-, di- or tri-xanthenyl derivatives depending upon the activity of the ring.

Phenol, when reacted with xanthydrol in acetic acid, with or without a condensing agent, gives 4-xanthenyl phenol (XLI) (40, 41, 42). Mizukami and Kanaya (43), upon studying this reaction more closely, isolated 2-xanthenyl phenol (XLII) as well as XLI from the reaction mixture. By employing an excess of xanthydrol, and heating the mixture on a water bath for 30 minutes, they recovered XLI, XLII and a third product which they assumed to be 2,4-dixanthenyl phenol (XLIII). When they treated XLI with xanthydrol, as above, they obtained 2,4,6-trixanthenyl

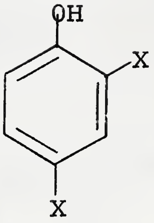
phenol (XLIV).



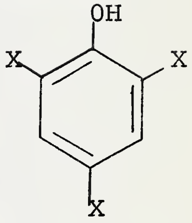
XLI



XLII



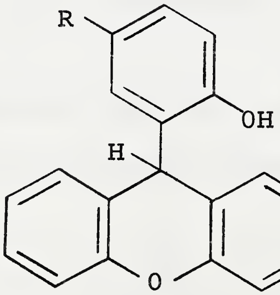
XLIII

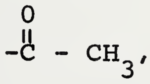


XLIV

X = 9-xanthenyl

When the para position in phenol is occupied, as in p-chlorophenol, p-hydroxyacetophenone, p-tert-($2^{\prime}$-phenylbutyl) phenol, p-tert-($2^{\prime}$-phenylamyl) phenol (40), p-cresol and p-hydroxybenzoic acid (44), the corresponding 2-xanthenyl-4- substituted phenols (XLV) are formed.





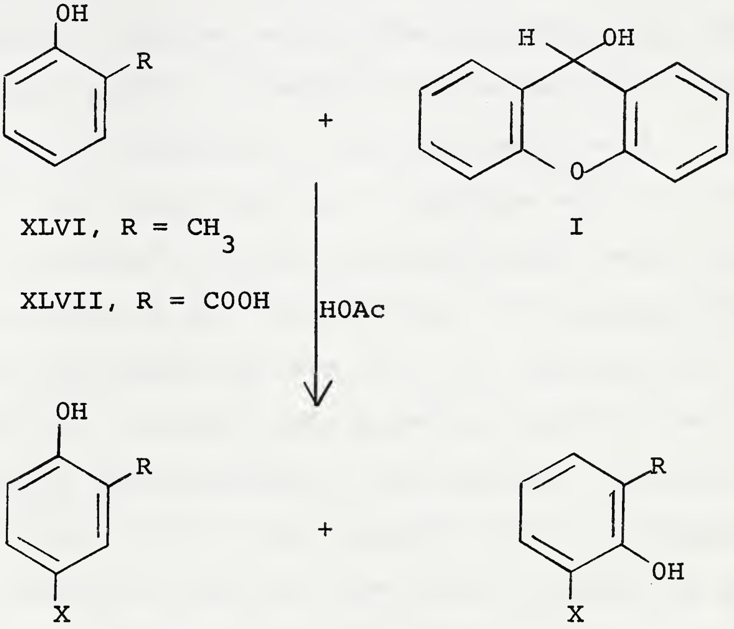
XLV, R = Cl, $-\overset{O}{\overset{\|}{C}} - CH_3$, etc.

[illegible]

[illegible]

[illegible]

The ortho substituted phenols o-cresol (XLVI) and o-hydroxybenzoic acid (XLVII) condense with xanthydrol (I) to yield a mixture of the corresponding 4-xanthenyl (XLVIII) and 6-xanthenyl (XLIX) 2-substituted phenols (equation 8).



XLVI, R = CH_3

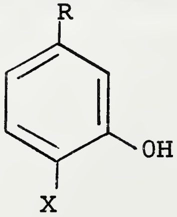
XLVII, R = COOH

I

(8)

XLVIII X = 9-xanthenyl XLIX

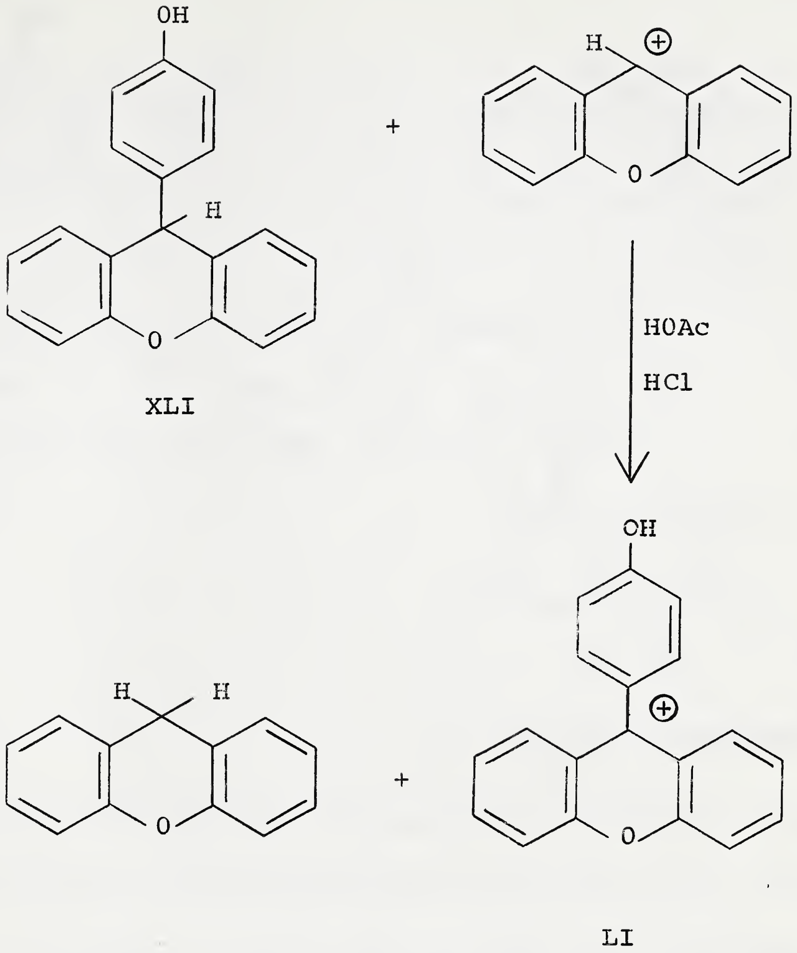
The corresponding meta compounds, on the other hand, yield only the 2-xanthenyl-5-substituted phenols (L) (44).



L, R = CH_3 or COOH, X = 9-xanthenyl

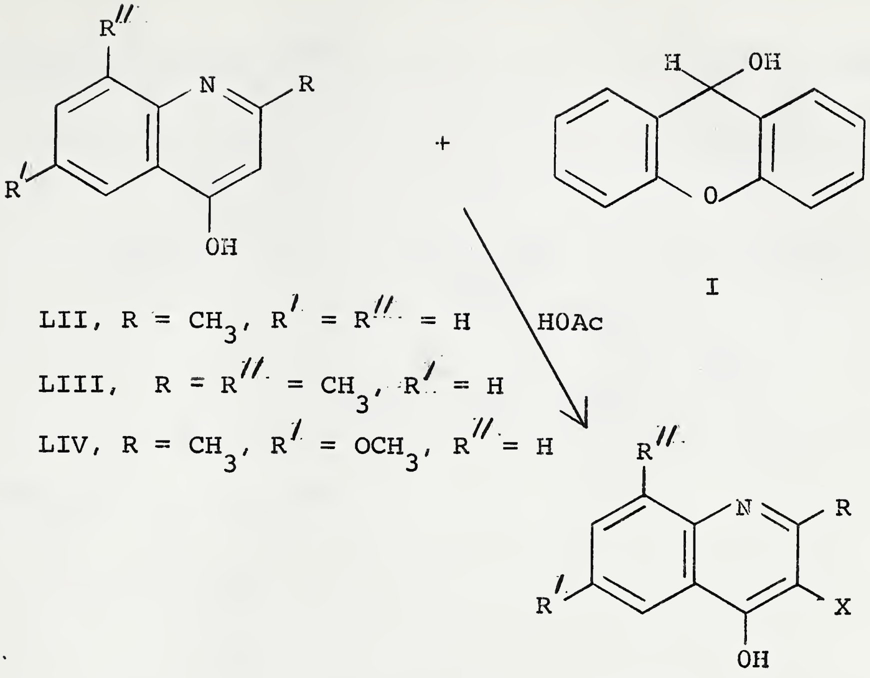
α-Naphthol also forms a monoxanthenyl derivative; however, no site of substitution was suggested (40).

The reaction of phenols with xanthydrol is accompanied by an intense coloration of the resulting reaction mixture. Lang (45) reported an intense red to brown coloration for ortho or meta substituted phenols (unsubstituted amines gave a blue to green color) with xanthydrol in glacial acetic acid. Yavorskii (46) studied the color reactions of phenols with an alcoholic solution of xanthydrol, containing hydrochloric acid, and found the colors produced to be characteristic of the phenol being tested. In a subsequent publication (47), he reported that the colors formed were unstable and therefore could not be used for the colorimetric determination of the phenols. Mizukami and Kanaya (48) studied this color reaction, but in a hydrochloric-acetic acid solution, and found the colors produced to be stable for periods up to 48 hours and suitable for the quantitative determination of a number of phenols. They attribute the color to the formation of the 9-(p-hydroxyphenyl) xanthylium salt (LI) of the phenol. This salt, they postulate, results from the reaction of the 4-xanthenyl phenol (XLI), which is the product originally formed in the reaction, with excess xanthylium ion (equation 9).



(9)

The hydroxyquinolines react similarly to the phenols, with xanthydrol, and form derivatives containing the 9-xanthenyl radical in the ortho or para position relative to the hydroxyl group (49). Thus, each of the following 4-hydroxyquinolines afforded the corresponding 3-xanthenyl derivative (LV): 2-methyl-(LII), 2,8-dimethyl-(LIII) and 2-methyl-6-methoxy-(LIV)-4-hydroxyquinoline.



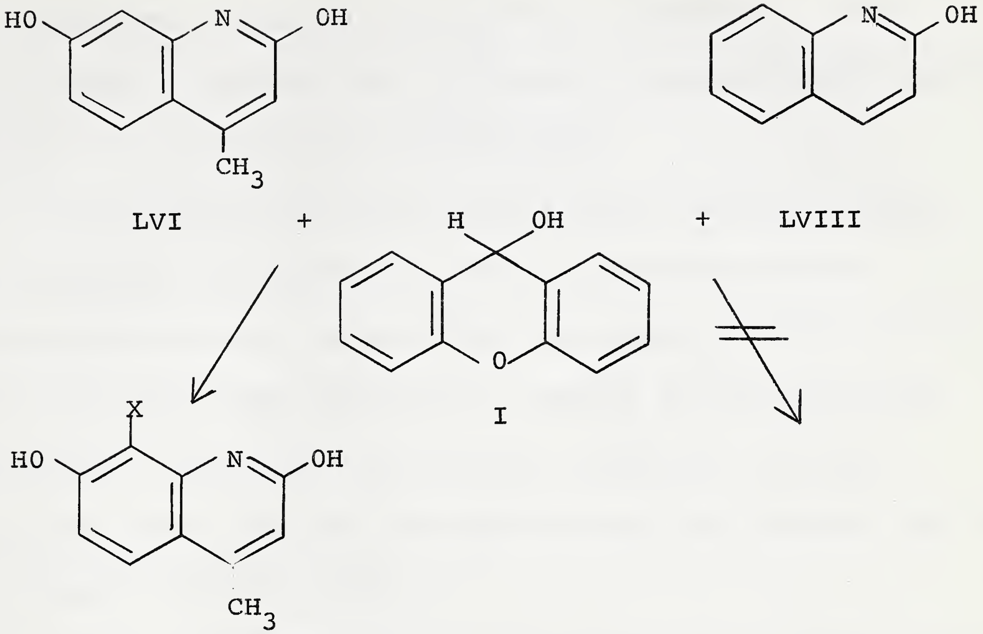
LII, $R = CH_3$, $R' = R'' = H$

LIII, $R = R'' = CH_3$, $R' = H$

LIV, $R = CH_3$, $R' = OCH_3$, $R'' = H$

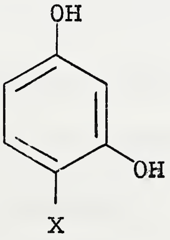
LV, X = 9-xanthenyl

3-Hydroxyquinoline gave the corresponding 4-xanthenyl compound, 5-hydroxyquinoline yielded the 8-xanthenyl derivative, whereas the 6- and 8- hydroxy isomers each afforded the 5-xanthenyl derivative. 2,7-Dihydroxy-4-methylquinoline (LVI) gave only the monosubstituted 8-xanthenyl derivative (LVII), while 2-hydroxyquinoline (carbostyril) (LVIII) failed to react at all with xanthydrol (I). The latter findings were attributed to the fact that the hydroxyl group, when situated in the 2-position of the quinoline nucleus, does not serve to activate the ring toward electrophilic substitution. Hydroxyquinolines containing alkylated or acylated hydroxyl groups did not react with xanthydrol.

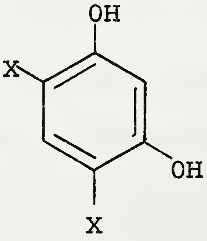

HO
N
OH
CH3
LVI
+
H
OH
+
LVIII
O
I
X
HO
N
OH
CH3

LVII, X = 9-xanthenyl

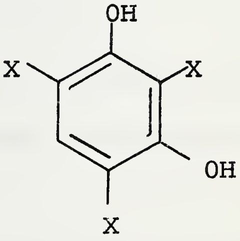
Resorcinol and xanthydrol in glacial acetic acid, at room temperature, yielded a mixture of 4-xanthenyl-(LIX) and 4,6-dixanthenyl-resorcinol (LX) (50, 51). Using excess xanthydrol, and heating the mixture for 30 minutes on a water bath, gave 2,4,6-trixanthenyl-resorcinol (LXI) (51).



LIX



LX



LXI

X = 9-xanthenyl

4-Nitro-and 4-chloro-resorcinol are alleged to yield the corresponding 2-xanthenyl derivatives. β-Resorcylic acid (2,4-dihydroxy-benzoic acid) afforded a monosubstituted compound; however, no structure was proposed (50).

Pyrocatechol and 4-chloropyrocatechol likewise yielded monoxanthenyl derivatives, as did 2-chlorohydroquinone. Hydroquinone itself, on the other hand, gave a mixture of a mono and dixanthenyl derivative. Several of the structures proposed by Fabre (50) for the above compounds are open to question, since they are not based on any real evidence, and some are almost certainly wrong.

1,3,5-Trimethoxy-(LXII) and 1,3,5-triethoxy-(LXIII) benzene condense readily with xanthydrol to yield the corresponding 1,3,5-trimethoxy (ethoxy)-2-xanthenyl-benzene (LXIV) (52).

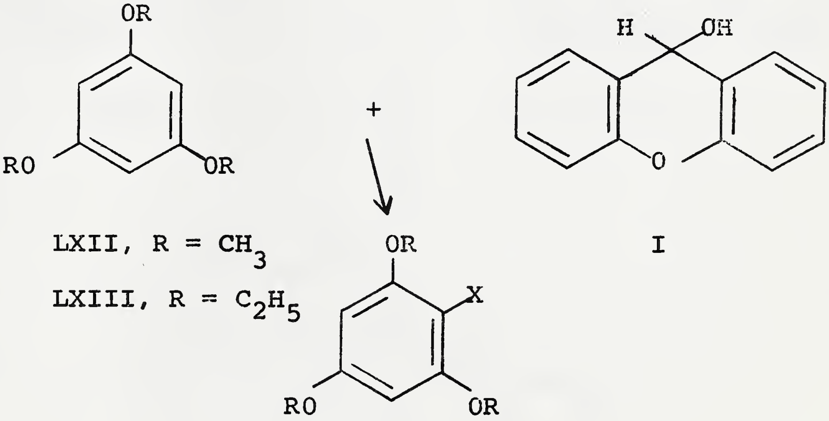
The amino group also activates the benzene ring toward electrophilic substitution with xanthydrol; however, as opposed to the hydroxyl group in phenols, substitution may occur on the amino nitrogen as well as on the activated ring (3).

Aromatic heterocyclic compounds condense with xanthydrol at the center or centers of high electron density within the heterocyclic molecule.

Pyrrole, when treated with xanthydrol, yielded 2,5-dixanthenylpyrrole (LXV) (5,53,54).

Illari (54), from a study with substituted pyrroles, found that xanthydrol reacted readily with pyrroles having free α

-positions to yield the corresponding dixanthenyl derivatives. If one of the α-positions was blocked, substitution occurred at the unoccupied α-position. Substitution of the nitrogen, as in N-phenyl-pyrrole, did not affect the reactivity of the two α-positions and N-phenyl-2,5-dixanthenyl pyrrole was the recovered product.



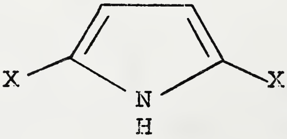
LXII, R = CH_3

LXIII, R = C_2H_5

I

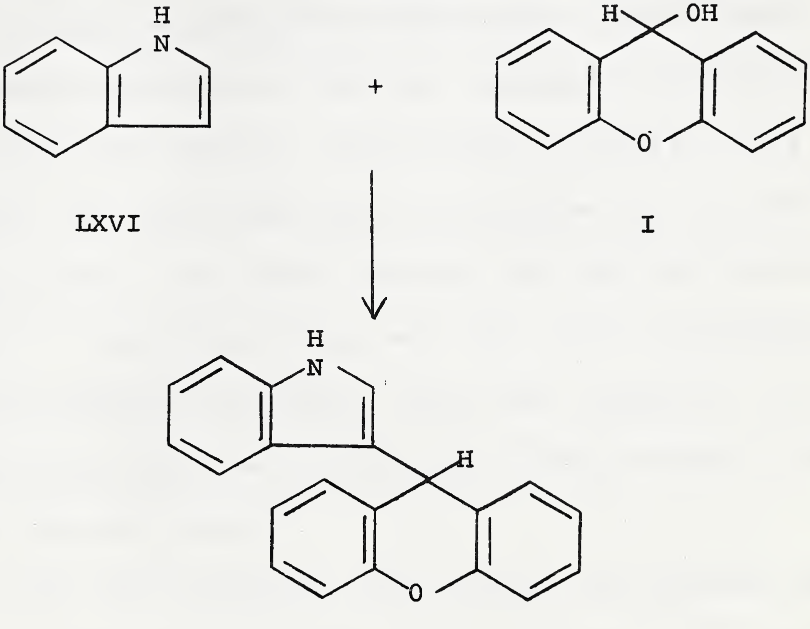
LXIV, X = 9-xanthenyl

A similar study with indoles (55) revealed that only monosubstitution occurred, preferably in the β-position; in the α-position if the β-position was occupied, and on the nitrogen if both the α- and β-positions were occupied. Thus, indole (LXVI) formed β-xanthenyl-indole (LXVII), α-methyl-indole gave α-methyl-



LXV, X = 9-xanthenyl

β-xanthenyl-indole, β-methyl-indole yielded α-xanthenyl-β-methyl-indole, and α-methyl-β-ethyl-indole afforded α-methyl-β-ethyl-N-xanthenyl-indole.

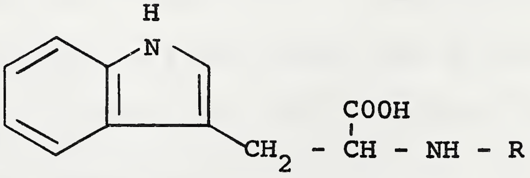


LXVI

I

LXVII

Dickman and Westcott (56), on the other hand, upon treating the amino acid dl-tryptophan (LXVIII) or its N-carbobenzoxy derivative (LXIX) with xanthydrol obtained dixanthenyl derivatives.



LXVIII, R = H

LXIX, R = cbz.

[illegible]

[illegible]

The dixanthenyl derivative of LXVIII could not be isolated, however, since they found that during the process of recrystallization from benzene-cyclohexane one of the xanthenyl groups was lost and only the monoxanthenyltryptophan was recovered. The dixanthenyl derivative of LXIX was isolated in the pure form from chloroform-ligroin. On the basis of Illari's (55) findings, they postulated that substitution had occurred in the 1,2-positions of the indole nucleus, but they had insufficient evidence to state which group was lost in the transformation to the monoxanthenyl-tryptophan. They felt, therefore, that one of the xanthenyl radicals was much more loosely bonded to the indole nucleus than the other.

As was true of phenols, the indoles also yielded highly colored solutions when treated with xanthydrol (55 , 57). Fearon (57) first observed the color formation with tryptophan, but he attributed this to the prior formation of indole from the amino acid. Dickman and Westcott (56) have since shown, as mentioned above, that tryptophan reacts directly with xanthydrol. Dickman and Crockett (58) adapted this color reaction to a quantitative photometric determination of tryptophan in blood plasma and in proteins.

The reactivity of xanthydrol with tryptophan has also been applied to the structural determination of proteins, in a process which Dickman calls "transxanthylation" (59). In this process, a protein such as insulin, lysozyme, ribonuclease (59) or cytochrome C (60) was

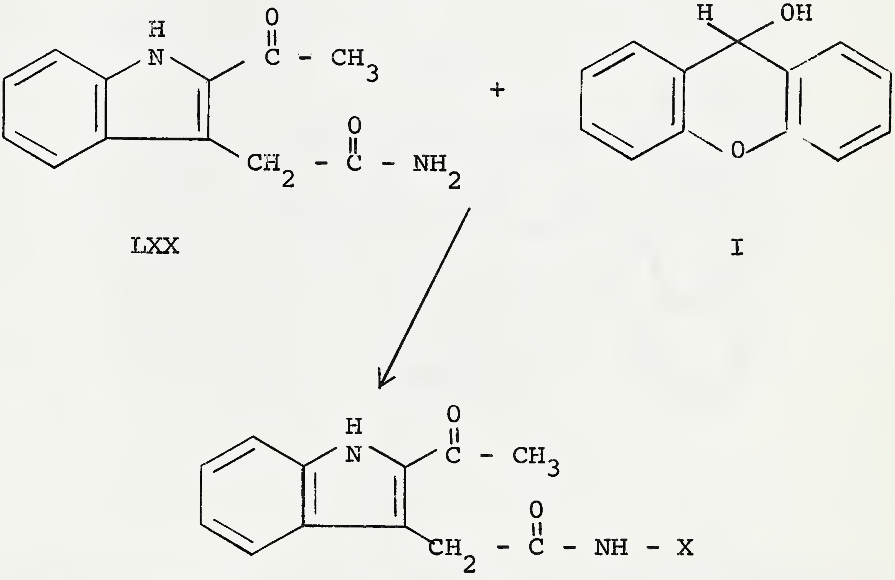
treated with xanthydrol resulting in a condensation of the latter with all of the reactive sites of the protein. (Xanthenyl substituted products have been isolated for each of the proteins mentioned above). Tryptophan was then added to the xanthenyl protein solution, causing the xanthenyl radicals to migrate to the indole nucleus of the tryptophan molecule, producing the colored dixanthenyl tryptophan whose concentration was then determined colorimetrically. This in turn permitted the calculation of the number of moles of xanthydrol taken up by the protein. In this manner, Dickman and coworkers found that insulin contained 2.2, lysozyme 9, ribonuclease 5.5 and cytochrome C 2.8 xanthenyl groups per mole of the protein. These. they point out, are minimum values since the so-called transxanthylation process may not be complete. This type of information could be useful in determining the active centers of proteins, once the identity of the amino acid side chains which react with xanthydrol have been definitely established.

Another practical application of the tryptophan-xanthydrol condensation has been made with respect to the recovery of DNP-amino acids from acid protein hydrolysates. Dickman and Asplund (61) found that they could increase the recoveries of DNP-lysines from DNP-lysozyme quite substantially if they treated the latter with xanthydrol prior to its acid hydrolysis. The increased recoveries were attributed to the formation of dixanthenyltrypophan in the intact protein, this compound having been shown to be stable in acid solution.

Fischer (62) devised a spot test with xanthydrol for the detection

of serotonin [5-hydroxy-3-(β-amino-ethyl) indole] in tumor tissue.

Finally, one other α, β-disubstituted indole has been condensed with xanthydrol; however, unlike the situation observed by Illari (55) substitution did not take place on the indole nitrogen. Plieninger et al. (63), upon reacting xanthydrol (I) with 2-acetyl-3-indolylacetamide (LXX), recovered only a monoxanthenyl derivative substituted at the amide nitrogen (LXXI).



LXXI, X = 9-xanthenyl

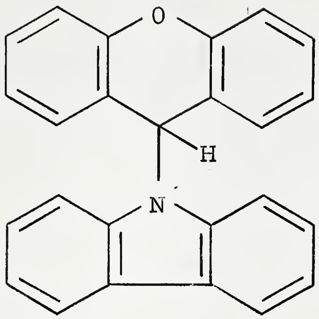
Carbazole, in which both the α and β positions are occupied is alleged to yield N-xanthenyl carbazole (LXXII) (55). Sawicki and Oliverio (5), however, questioned this structure (LXXII) since they found that N-methyl-carbazole also gave a monoxanthenyl derivative. As N-alkylated carbazoles are known to be attacked mainly in the 3-position by electrophilic reagents, and from ultraviolet data, they assigned the corresponding structure (LXXIII)

[illegible]

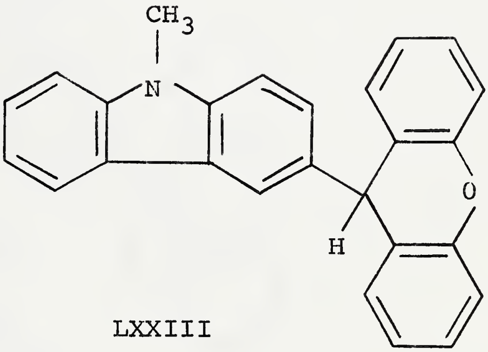
[illegible]

[illegible]

to the product.

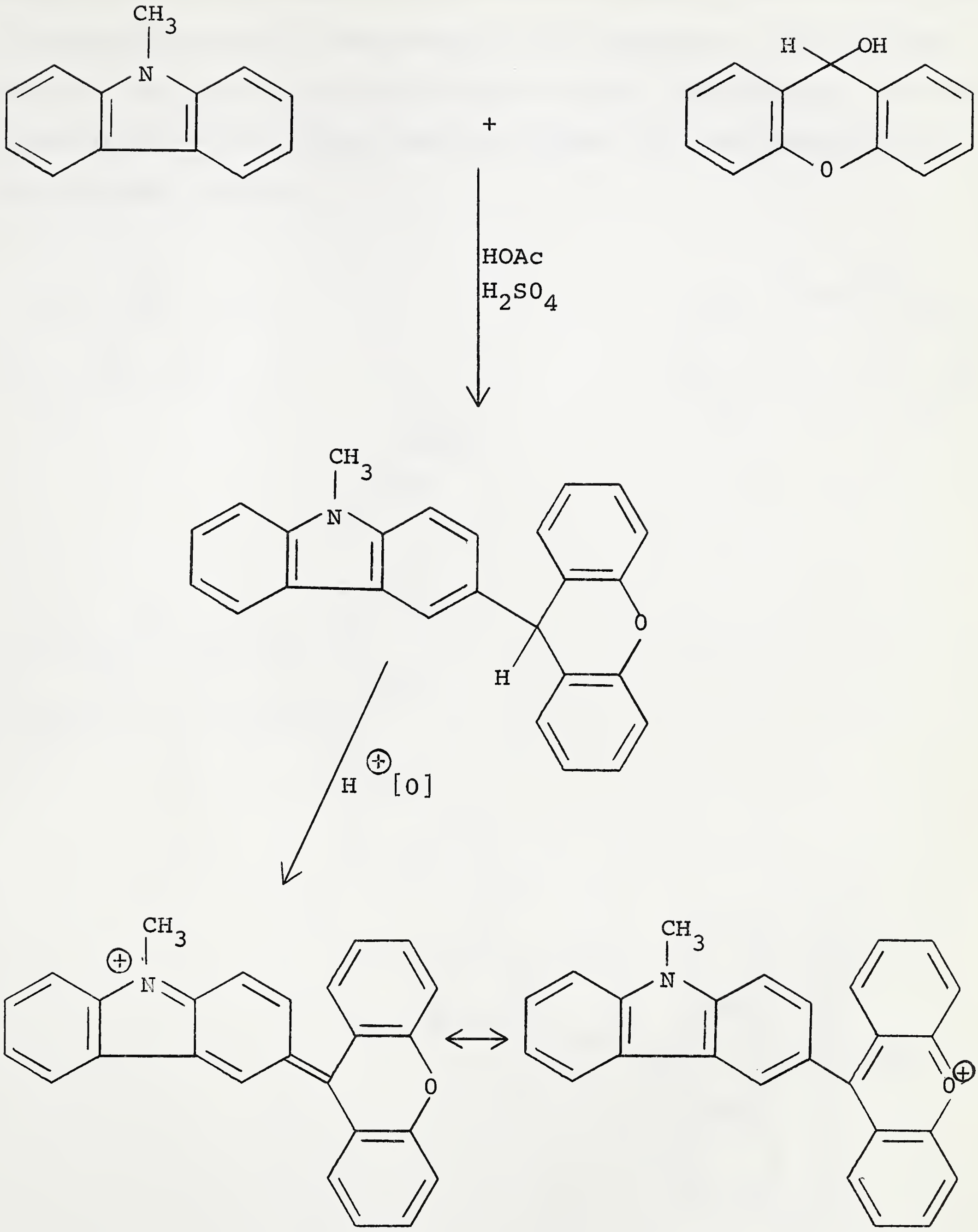


LXXII



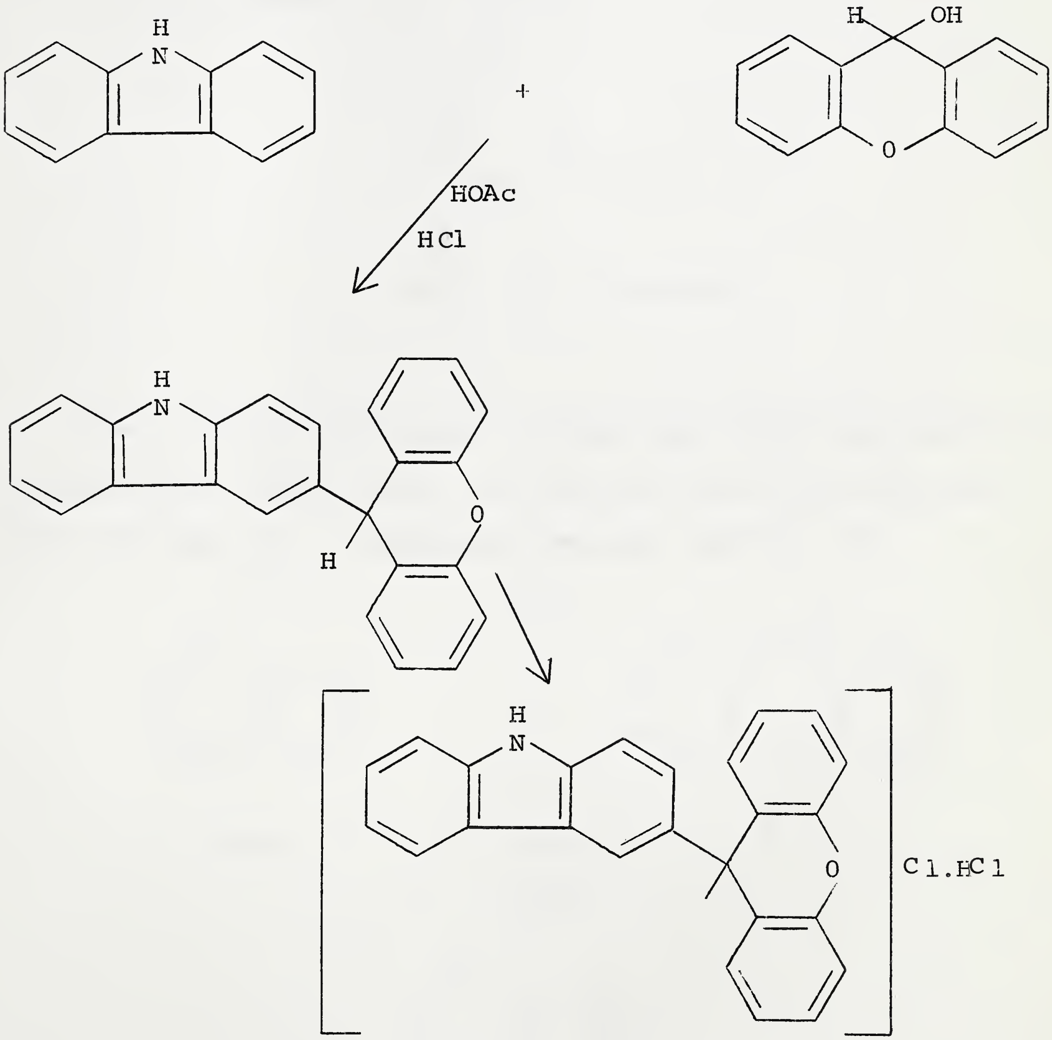
LXXIII

The carbazoles also yielded colored solutions with xanthydrol. Arreguine (64) first reported the formation of a deep indigo-blue color when carbazole was treated with a reagent composed of xanthydrol in acetic-hydrochloric acid. Sawicki and Oliverio (5), examined the colors formed by a large number of substituted carbazoles, and other active hydrogen compounds, with both xanthydrol and thiaxanthydrol in acetic-sulfuric acid solution. For the color reaction between N-methyl-carbazole and xanthydrol they postulated the following reaction scheme:


+
HOAc
H_2SO_4
$H^{\oplus}$ [O]

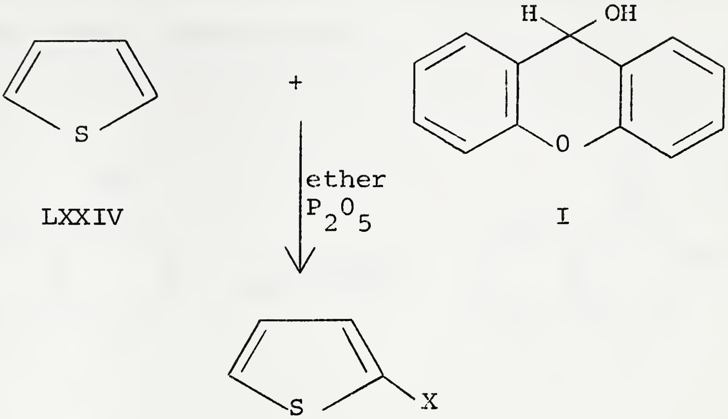
Gilbert and coworkers (65), seemingly unaware of the work of either Illari (55) or Sawicki and Oliverio (5), utilized the

color reaction between carbazole and xanthydrol as the basis for a spectrophotometric determination of carbazole. They carried out the reaction using hydrochloric rather than sulfuric acid and postulated the following reaction scheme for the production of the colored product:



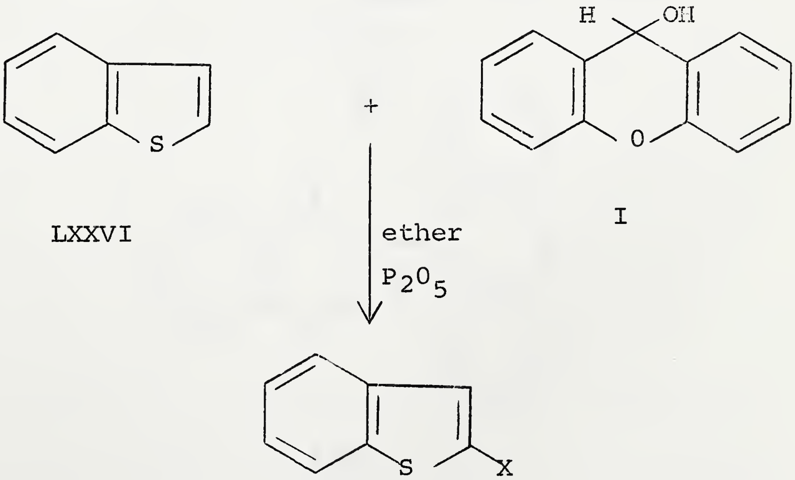
As opposed to pyrrole, thiophene (LXXIV), when heated with xanthydrol (I) in ether, in the presence of phosphorus pentoxide,

yielded only the mono-α-xanthenyl derivative (LXXV).


H
OH
S
O
+
ether
P_2O_5
LXXIV
I
S
X

LXXV, X = 9-xanthenyl

Also, unlike indole which yielded the β-xanthenyl derivative, thianaphthene (LXXVI) under the reaction conditions described above afforded the α-xanthenyl compound (LXXVII) (66).


H
OH
S
O
+
ether
P_2O_5
LXXVI
I
S
X

LXXVII, X = 9-xanthenyl

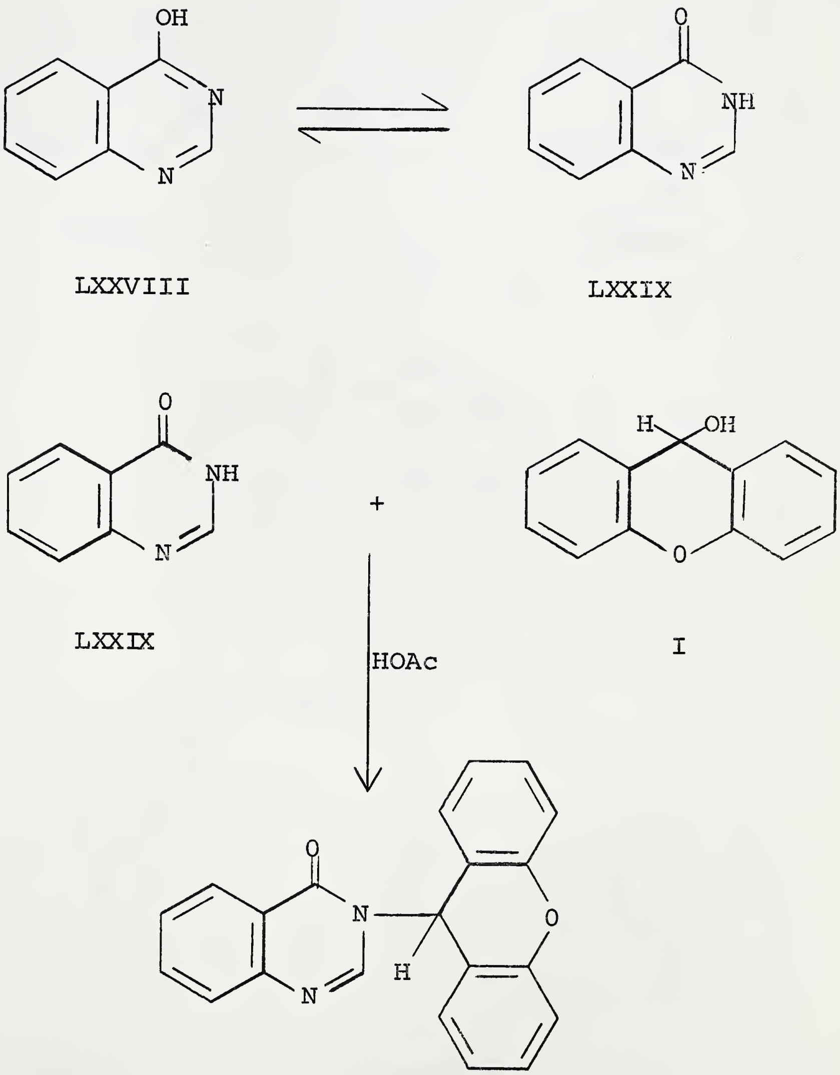
[illegible]

[illegible]

[illegible]

[illegible]

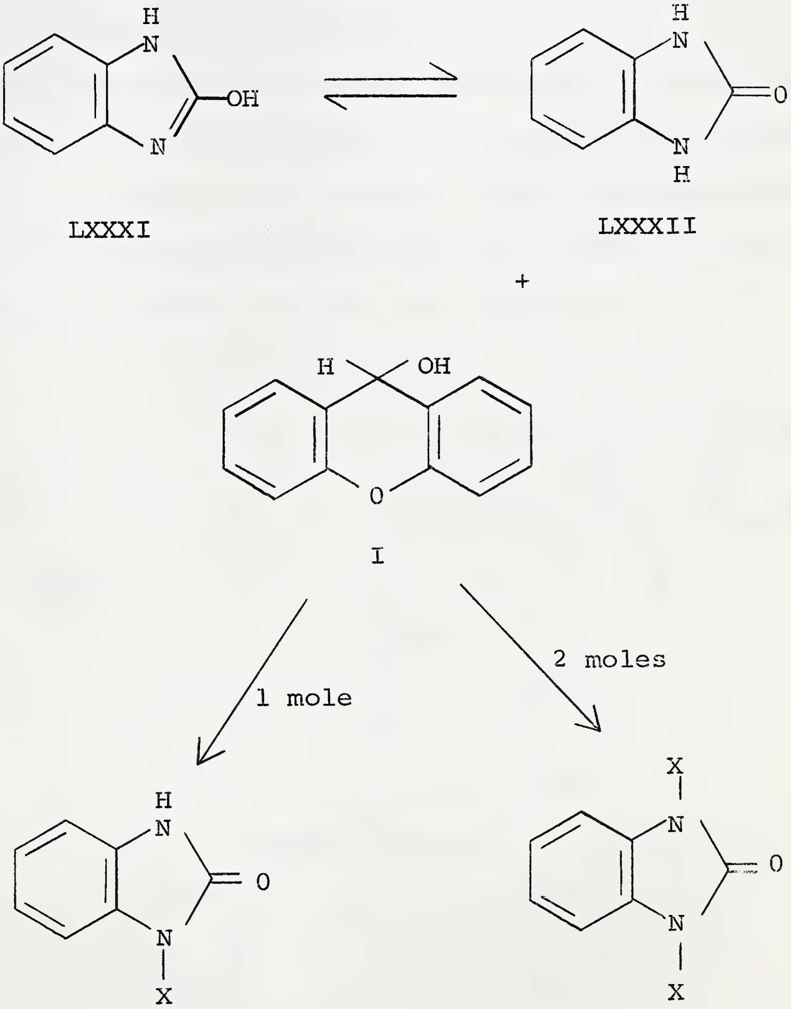
4-Hydroxyquinazoline (LXXVIII) reacted in its tautomeric keto form (LXXIX), with xanthydrol (I), yielding 3-xanthenyl-4-quinazolone (LXXX) (equation 10).


OH
N
N
O
NH
N
LXXVIII
LXXIX
O
NH
N
+
H
OH
O
LXXIX
HOAc
I
O
N
N
H
O

(10)

LXXX

2-Hydroxybenzimidazole (LXXXI) also condensed from its keto form (LXXXII) with an equimolar amount of xanthydrol (I) to give N-xanthenyl-benzimidazolone (LXXXIII), and with 2 moles of I to yield N,N^1-dixanthenylbenzimidazolone (LXXXIV) (equation 11).



LXXXI LXXXII

I (11)

LXXXIII X = 9-xanthenyl LXXXIV

2-Mercaptobenzimidazole behaves similarly to the 2-hydroxy -

[illegible]

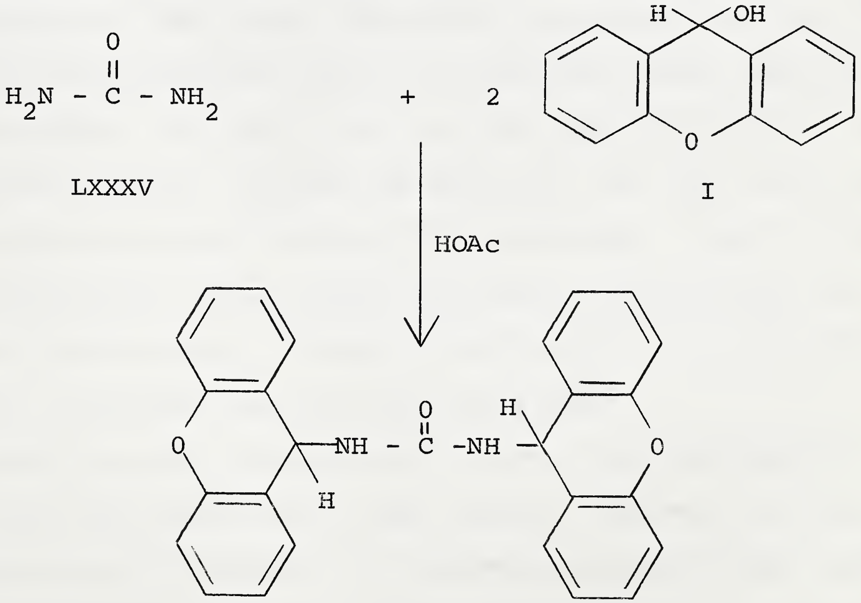
[illegible]

[illegible]

analog (67).

One other aromatic compound has been shown to react with xanthydrol; however, the derivative was not isolated. Sawicki _et al._ (68) found that azulene condensed with xanthydrol to give a colored solution which they employed as the basis for its spectrophotometric determination.

Perhaps the best known of the reactions of xanthydrol (I) are those involving condensation at a hydrogen-substituted nitrogen atom. Undoubtedly the best known individual example from this group is the reaction with urea (LXXXV) to give N,N^1-dixanthenylurea (LXXXVI) (5, 69, 70) (equation 12).



LXXXVI

(12)

The interesting feature about this reaction is the fact that it proceeds quantitatively, and consequently it has been employed

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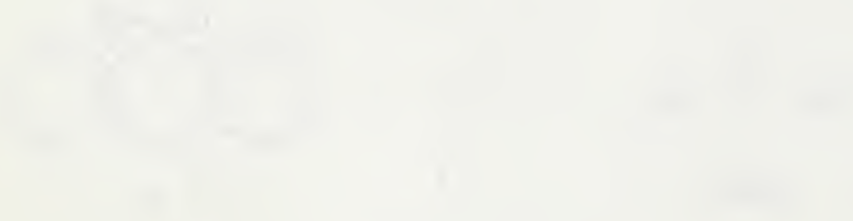

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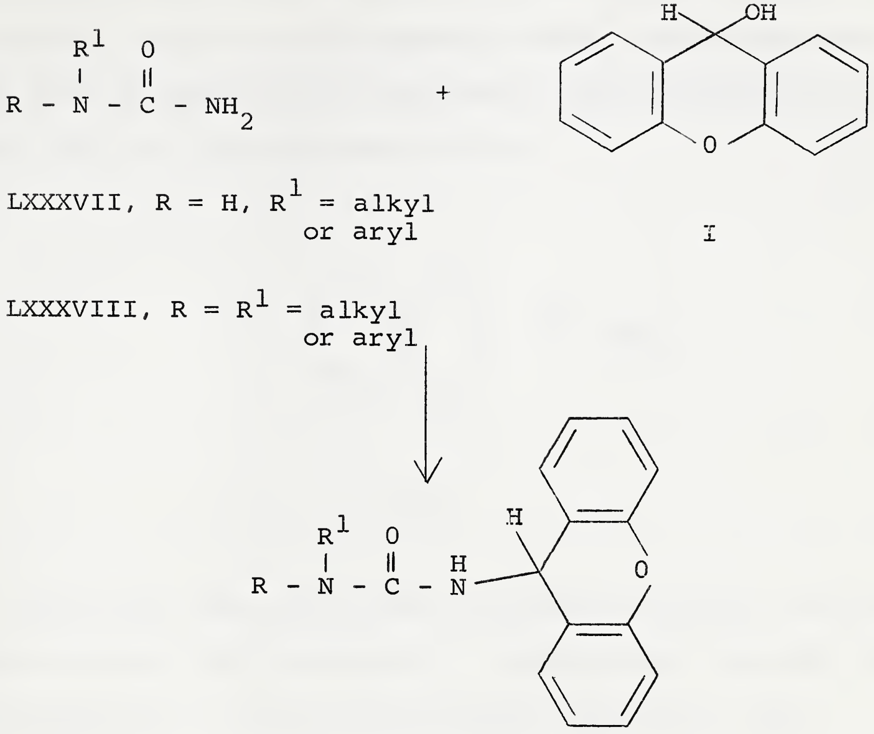
extensively for the quantitative determination of urea. The latter has been achieved either gravimetrically (71, 72, 73), or volumetrically by oxidation of the dixanthenylurea with excess iodic acid (74, 75), potassium permanganate (76) or potassium dichromate (8, 77). A visual turbidity test (78, 79, 80) and a microscopic examination (81) of the dixanthenylurea precipitate have been employed for the diagnosis of a suspected uremic state in patients. Zinsser and Cohen (82) used a resin treated with xanthydrol to remove urea, in their study of the capacity of resins to simulate chemical processing by the kidney. Finally, Graff et al. (13) made use of the reaction of xanthydrol with urea for the quantitative determination of arginine in protein hydrolysates. They first carried out an enzymic cleavage of arginine with arginase, which converted the former quantitatively into ornithine and urea. The urea was then estimated by the xanthydrol method. Dickman and Westcott (56) have since shown that arginine reacts directly with xanthydrol. Their evidence showed that the α-amino group was intact and therefore not the site of substitution. However, the derivative was not isolated nor was a possible reaction site suggested.

Alkyl or aryl substitution of one of the hydrogens in urea destroyed the reactivity at that center. Adriani (3) found that monosubstituted (LXXXVII) or asymmetric disubstituted (LXXXVIII) ureas formed monoxanthenyl derivatives (LXXXIX), whereas symmetric disubstituted ureas (XC) failed to condense with xanthydrol (I).

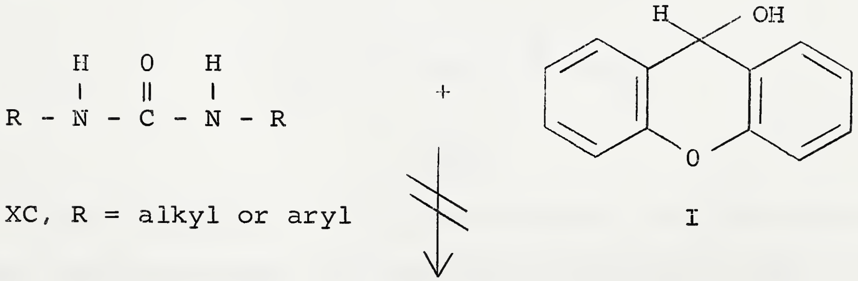


LXXXVII, R = H, R^1 = alkyl or aryl

I

LXXXVIII, R = R^1 = alkyl or aryl

LXXXIX

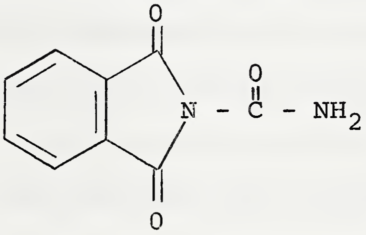


XC, R = alkyl or aryl

I

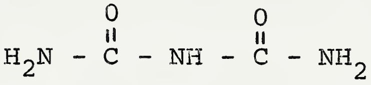
In line with these findings, Fosse (53) had previously obtained a monoxanthenyl derivative with phenylurea, and Sawicki and Oliverio (5) have since prepared the monoxanthenyl derivative of p-ethoxyphenylurea.

Hahn et al. (83) utilized the above behavior of substituted ureas in proving the structure of phthaloyl-urea (XCI). Aside from other evidence, the compound formed a monoxanthenyl derivative with xanthydrol and consequently they assigned the structure shown (XCI) to this urea derivative.



XCI

Thioureas have been shown to behave similarly to the ureas when condensed with xanthydrol. Carbamylurea (biuret) (XCII) is also reported to yield a dixanthenyl derivative (53).

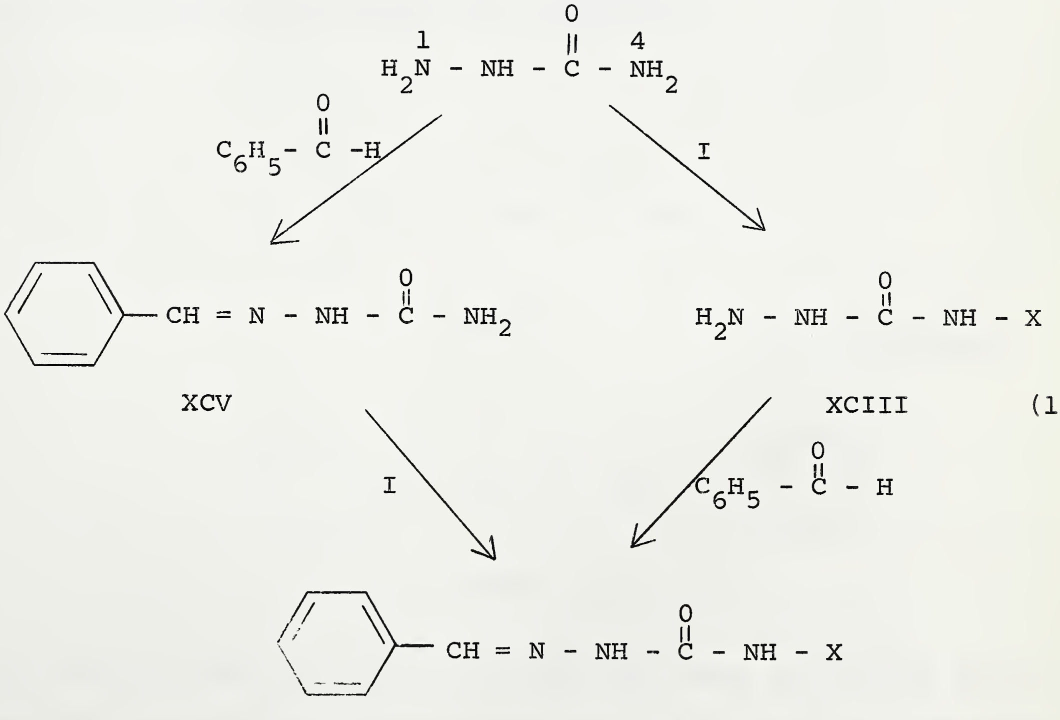


$$H_2N - \overset{O}{\overset{\|}{C}} - NH - \overset{O}{\overset{\|}{C}} - NH_2$$

XCII

Substitution of one of the hydrogens in urea with an amino group (semicarbazide) also destroys activity at that nitrogen, however, the nitrogen of the added amino group becomes reactive toward xanthydrol. Fosse (25) first reported that the condensation of semicarbazide with xanthydrol resulted in a monoxanthenyl derivative, but no mention was made of the point of attack. Doucet (84) studied this reaction more closely and found that using

equimolar quantities of reactants he also recovered a monosubstituted product, but when he used 2 moles or more of xanthydrol per mole of semicarbazide he recovered a dixanthenyl derivative. By employing intermediate quantities of xanthydrol he got a mixture of the two products. He then proved that the monosubstituted product was 4-xanthenylsemicarbazide (XCIII) since the derivative still formed a semicarbazone with benzaldehyde (XCIV) (or acetone), showing that the 1-position in semicarbazide had not been substituted. Additional proof came from the fact that the semicarbazone of benzaldehyde (XCV) condensed with xanthydrol (I) to yield a product (XCIV) identical with that obtained from the condensation of XCIII with benzaldehyde (equation 13).


1
4
H2N - NH - C(=O) - NH2
C6H5 - C(=O) - H
I
CH = N - NH - C(=O) - NH2
H2N - NH - C(=O) - NH - X
XCV
XCIII
I
C6H5 - C(=O) - H
CH = N - NH - C(=O) - NH - X

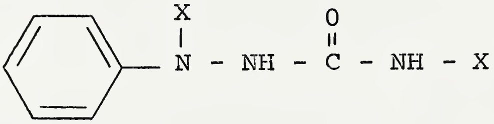
(13)

XCIV

X = 9-xanthenyl

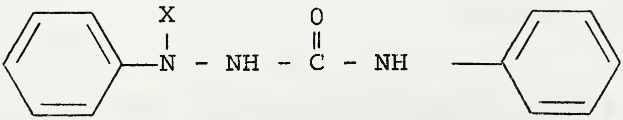
Since 4-phenyl- and 4,4-diphenyl-semicarbazide both formed monoxanthenyl derivatives, they therefore concluded that the disubstituted product must be 1,4-dixanthenyl-semicarbazide.

1-Phenyl-semicarbazide, on the other hand, gave not only a monosubstituted product but a dixanthenyl derivative as well. 1,4-diphenyl-semicarbazide likewise afforded a monosubstituted product. To account for the unexpected behavior of these compounds, Doucet suggested that when a hydrogen atom in the 1-position is substituted by a phenyl group, the remaining hydrogen can then be substituted by the xanthenyl radical. Accordingly, he assigned 1,4-dixanthenyl-1-phenyl-semicarbazide (XCVI) as the structure of the disubstituted derivative, and 1-xanthenyl-1,4-diphenylsemicarbazide (XCVII) as the product from the reaction of 1,4-diphenylsemicarbazide with xanthydrol.



XCVI

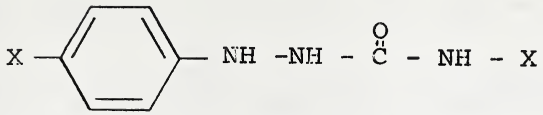
X = 9-xanthenyl



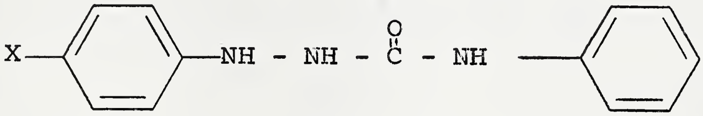
XCVII

He, however, does not offer any proof for these structures and it may well be that ring substitution has in fact occurred (analogous to Adriani's (3) findings with amines). Thus, the following

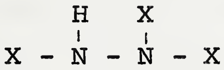
structures are also possibilities for these compounds:



X = 9-xanthenyl



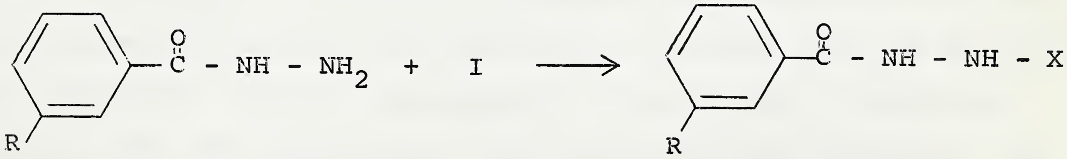
Hydrazine is reported to yield trixanthenylhydrazine (XCVIII) when condensed with xanthydrol in dilute acetic acid (85).



$$X - \overset{\overset{H}{|}}{N} - \overset{\overset{X}{|}}{N} - X$$

XCVIII, X = 9-xanthenyl

Benzoyl hydrazine (XCIX) (84), and m-nitrobenzoyl hydrazine (C) (5), however, yielded only the corresponding monoxanthenyl derivatives (CI).



XCIX, R = H

C, R = NO_2

CI, X = 9-xanthenyl

Fosse and Hieuelle (85) utilized the reactivity of hydrazine with xanthydrol (I) to demonstrate the existence of the hydrazone of glyoxylic acid in aqueous solution (although the hydrazone has never been isolated in the pure state) by precipitating dixanthenyl-hydrazonoglyoxylic acid (CII) from the

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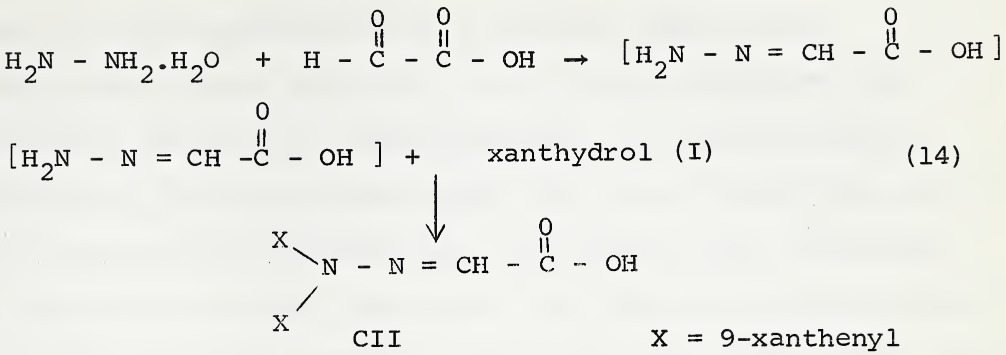
solution with xanthydrol (I) (equation 14).



$$H_2N-NH_2\cdot H_2O + H-\overset{O}{\overset{\|}{C}}-\overset{O}{\overset{\|}{C}}-OH \rightarrow [H_2N-N=CH-\overset{O}{\overset{\|}{C}}-OH]$$

$$[H_2N-N=CH-\overset{O}{\overset{\|}{C}}-OH] + \text{xanthydrol (I)} \qquad (14)$$

$$\downarrow$$

$$X_2N-N=CH-\overset{O}{\overset{\|}{C}}-OH$$

CII

X = 9-xanthenyl

Hydroxylamine is also reported to yield a monoxanthenyl derivative (25).

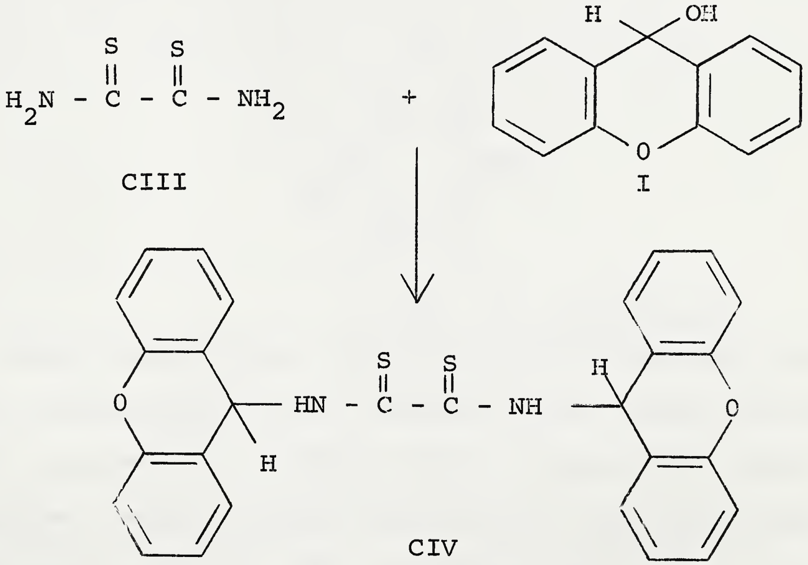
Primary amides condense with xanthydrol to give the corresponding monoxanthenyl derivatives (3,4,53,56,63,69,70,86). As was the case with ureas, substitution of one of the amide hydrogens resulted in a loss of reactivity (3,4). Compounds containing two amide groupings, such as malonamide and succinamide, yielded the corresponding dixanthenyl derivatives (53).

Several amino acids having an unsubstituted amide group have been reacted with xanthydrol. Asparagine and glutamine have both been shown to condense with xanthydrol, although only the derivative of the former compound was actually isolated (56). Shimonishi et al. (87) took advantage of the reactivity with glutamine, and employed the xanthenyl moiety as a protecting group during the synthesis of peptides containing this amino acid as the C-terminal group. The xanthenyl group was later easily removed by treatment with hydrogen bromide in glacial acetic acid.

Bredereck and coworkers (86) prepared several xanthenyl derivatives of amides by simply fusing xanthydrol with the amide. Among these was the xanthenyl derivative of formamide which

Phillips and Pitt (4) had previously attempted to prepare, without success, by the standard method in glacial acetic acid.

Thioamides behave similarly to the oxygen analogues when treated with xanthydrol. Thioacetamide (27), thiobenzamide, p-methyl- and p-methoxythiobenzamide (86) each afforded the corresponding monoxanthenyl derivative. The latter three thioamides were condensed in xylene, employing zinc chloride as the condensing agent. Thioformamide, however, failed to react under these conditions. Dithiooxamide (CIII) reacted with xanthydrol (I) in warm glacial acetic acid to yield N,N^1-bis(9-xanthenyl) dithiooxamide (CIV). Oxamide, on the other hand, failed to yield a satisfactory derivative because of its insolubility in glacial acetic acid (27).



Other molecules containing the amide functional grouping such as the urethanes (3,5,53,70,88), and allophanates (89) reacted in a similar manner with xanthydrol.

Cyclic imides also possess an active hydrogen atom and condense

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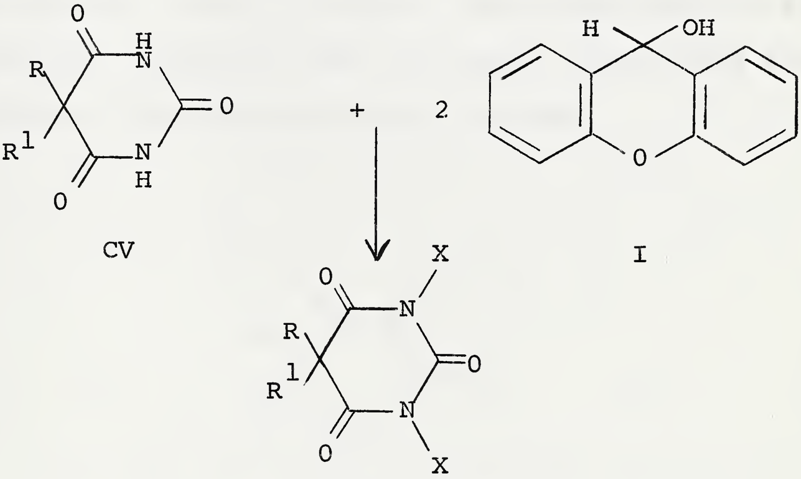
readily with xanthydrol to give the corresponding N-xanthenyl derivatives. Examples of such compounds that have been condensed with xanthydrol include succinimide (53), oxazolidinedione (90), rhodanine (67,91), 5-akylidene rhodanines (91), and saccharin (1,37).

The barbiturates (5,5-disubstituted barbituric acids) (CV), which are cyclic diimides, condense with xanthydrol (I) to give the corresponding 1,3-dixanthenyl barbiturates (CVI) (37,70, 92,93,94,95,96).



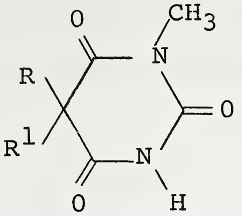
CV

I

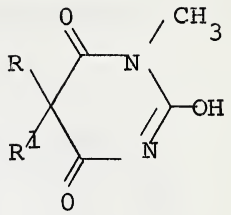
CVI, X = 9-xanthenyl

Substitution of one of the nitrogen atoms by a methyl group as in hexobarbital (CVII), mephobarbital (CVIII), or metharbital (CIX) resulted in a complete loss of reactivity rather than affording a monoxanthenyl derivative as one might expect. McCutcheon and Plein (94) attributed this lack of reactivity to similar results with substituted amides; however, the validity or applicability of this analogy is questionable. To explain these findings, one would have to assume that these N-methyl substituted barbiturates condense from their tautomeric enol form (CX) in the reaction solvent, and show little or no tendency to react from

the triketonic state.

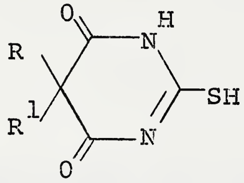


CVII, R = CH_3, R^1 = 1 - cyclohexen-1-yl
CVIII, R = C_2H_5, R^1 = C_6H_5
CIX, R = R^1 = C_2H_5



CX

Diethylthiobarbituric acid (CXI) (97) and thiopental (CXII)(94) are reported to yield only monoxanthenyl derivatives. No explanations are given; however, again it appears that these barbiturates must also react from their diketonic form (as shown) which would account for the formation of monosubstituted derivatives.

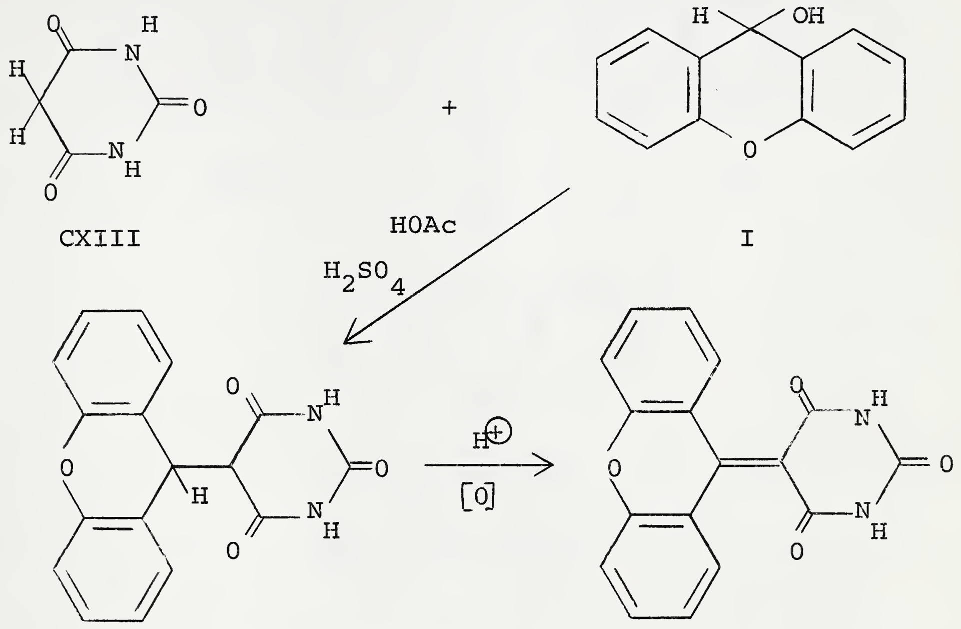


CXI, R = R^1 = C_2H_5

CXII, R = C_2H_5, R^1 = $-CH(CH_3)-CH_2-CH_2-CH_3$

Barbituric acid (CXIII) itself, when reacted with xanthydrol in glacial acetic acid gives a brilliant purple color, and a precipitate which could not be purified (94). No similar color formation accompanied the reaction with 5,5-disubstituted barbituric acids. Sawicki and Oliverio (5) examined this color reaction in an acetic-sulfuric acid solution and since, under

these conditions, the 5,5-disubstituted barbituric acids did not give this color they postulated the following structure for the barbituric acid-xanthydrol color product (CXIV):



CXIII

I

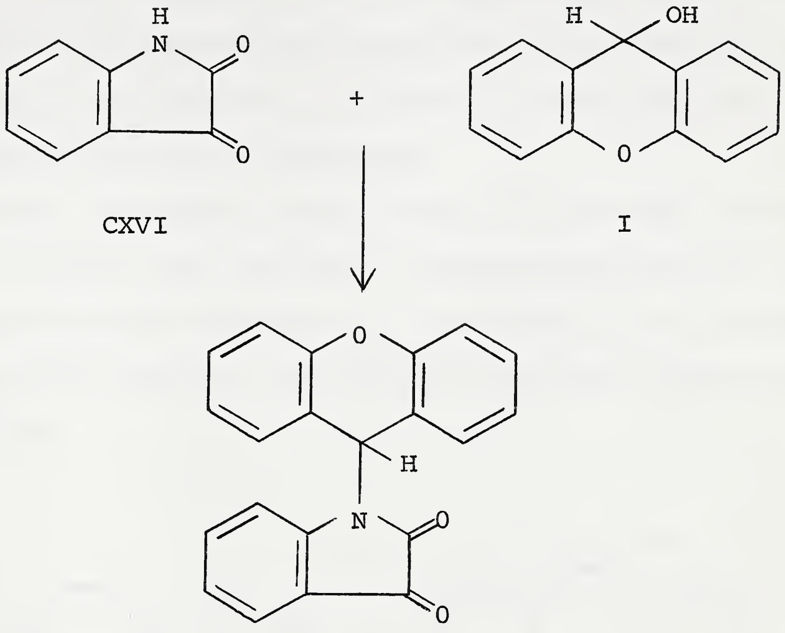
CXV

CXIV

However, since they report that the sulfuric acid was necessary as an oxidizing agent, to convert (CXV) to the colored species (CXIV), this would not explain the color reaction obtained by Phillips and Pitt who did not employ any sulfuric acid. Furthermore, Dalev and Gagauzov (98) have since reported a color formation with 5-mono-substituted barbituric acids and xanthydrol, which would make a structure of the type CXIV impossible for these colored products. Therefore, although it appears that xanthydrol condenses with barbituric acid at the active 5-position, the structure of the colored species would seem to be in doubt.

Isatin (CXVI) is reported to behave similarly to the

α, β-disubstituted indoles, in that it also afforded the N-xanthenyl derivative (CXVII) (55).

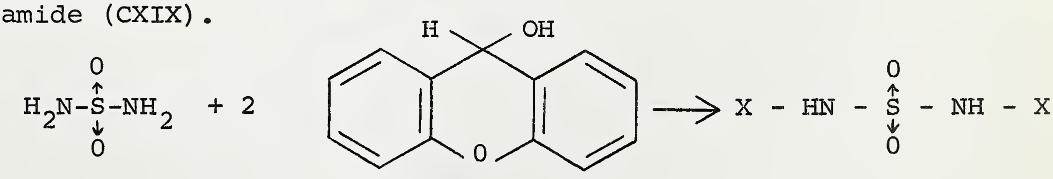


CXVI I

CXVII

Because of the reactivity of xanthydrol (I) with carboxylic acid amides, an extension to the sulfonic acid amides (sulfonamides) soon followed. (1,5,70,99,100,101,102).

Wood (99) in 1935, first reported the reaction with the analog of urea, sulfamide (CXVIII), to give N,N^1-dixanthenylsulfamide (CXIX).



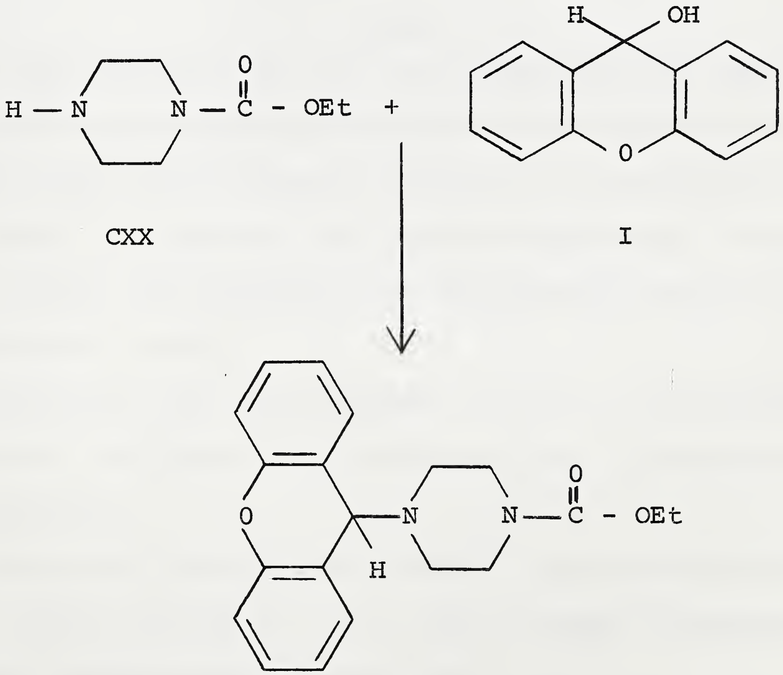
$$H_2N-\overset{O}{\underset{O}{S}}-NH_2 + 2\,\mathrm{I} \longrightarrow X-HN-\overset{O}{\underset{O}{S}}-NH-X$$

CXVIII I CXIX

X = 9-xanthenyl

He also condensed some benzene sulfonamides with xanthydrol, however, no experimental details were given. Phillips and Frank (1) then undertook a more thorough study of the reaction of benzenesulfonamides with xanthydrol and as mentioned in part 1 of the introduction, arrived at conclusions similar to those found with the carboxyamides.

Finally, one other condensation at a nitrogen atom has been reported, namely that involving 1-carbethoxypiperazine (CXX). Refluxing CXX with xanthydrol (I) in toluene, in the presence of acetic acid, yielded 1-carbethoxy-4-xanthenyl-piperazine (CXXI) (103).



CXX

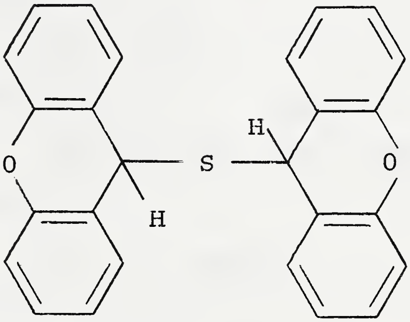
I

CXXI

The last of the reaction types of xanthydrol, referred to earlier, are those involving condensation at a hydrogen-substituted

sulfur atom to yield the corresponding S-xanthenyl derivatives.

Fosse (104) treated xanthydrol dissolved in acetic acid with hydrogen sulfide, and obtained dixanthenyl sulfide (CXXII). [The formula of the latter is erroneously represented as the disulfide by Wawzonek (22)].

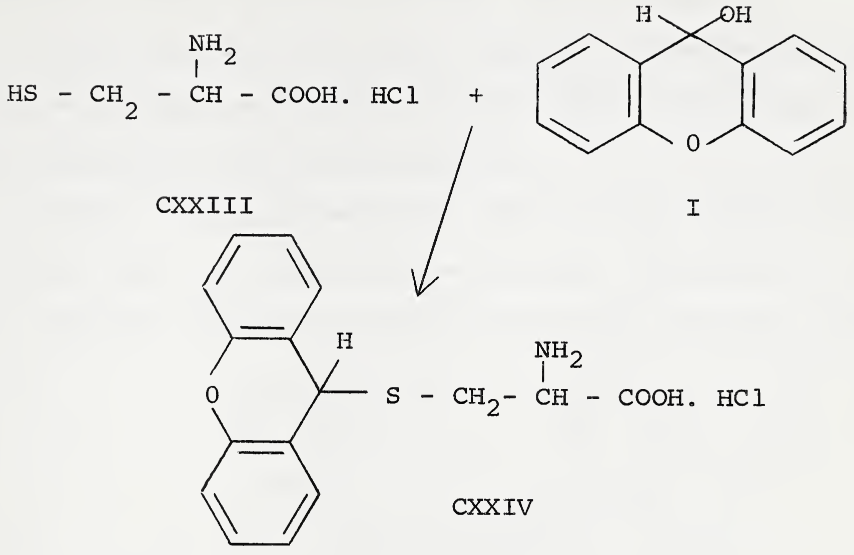


CXXII

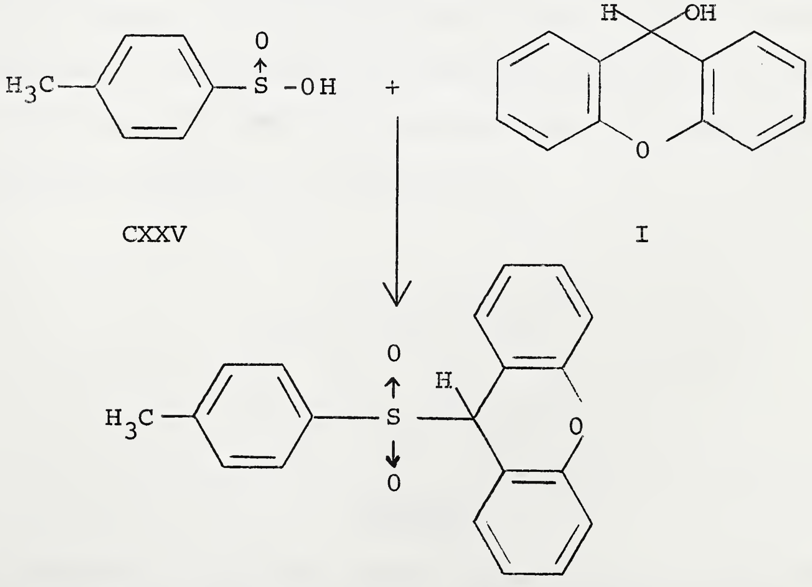
Sawicki and Oliverio (5) have also prepared this compound, as well as the corresponding derivative from hydrogen selenide. Besides these, they prepared a series of S-xanthenyl derivatives of a number of aliphatic and aromatic mercaptans. With all but one of these, they prepared the corresponding derivatives with thiaxanthydrol (XII).

Pellerano (105) synthesized a series of S-xanthenyl derivatives of 2-thio-and 4-thioquinolines in ethanol-acetic acid solution.

L-Cysteine hydrochloride (CXXIII) when heated with xanthydrol (I) in acetic acid at 65° for 1 hour yielded S-xanthenyl-L-cysteine hydrochloride (CXXIV) (56).


NH2
HS - CH2 - CH - COOH. HCl
+
H
OH
O
CXXIII
I
H
O
S - CH2- CH - COOH. HCl
NH2
CXXIV

Toluene-p-sulfinic acid (CXXV) and its sodium salt condense readily with xanthydrol to give p-tolyl-xanthenyl sulfone (CXXVI). (52,106,107).

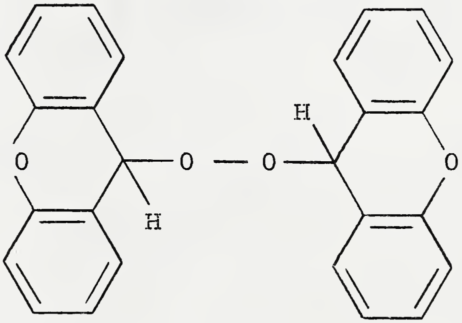

H3C
O
S -OH
+
H
OH
O
CXXV
I
O
S
O
H
O
H3C

CXXVI

Hookway (108) recovered this product (CXXVI) from the reaction of p-tolyl-toluene-p-thiol sulfonate with xanthydrol.

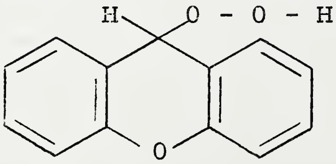
Aside from the three main types of condensation reactions which xanthydrol undergoes, several other interesting reactions with xanthydrol have been reported.

Fosse (104) reported that under nearly similar conditions to those used for obtaining the sulfide, treatment of xanthydrol with hydrogen peroxide yielded dixanthenyl peroxide (CXXVII).



CXXVII

Davies et al. (29) studied this reaction more closely, and found that using either 90% hydrogen peroxide in ether, or neutral 30% aqueous hydrogen peroxide they obtained xanthenyl-hydroperoxide (CXXVIII) in good yield.



CXXVIII

In the presence of glacial acetic acid, however, the hydroperoxide (CXXVIII) was readily transformed to the dixanthenyl peroxide

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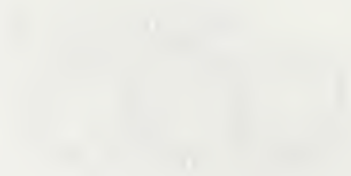

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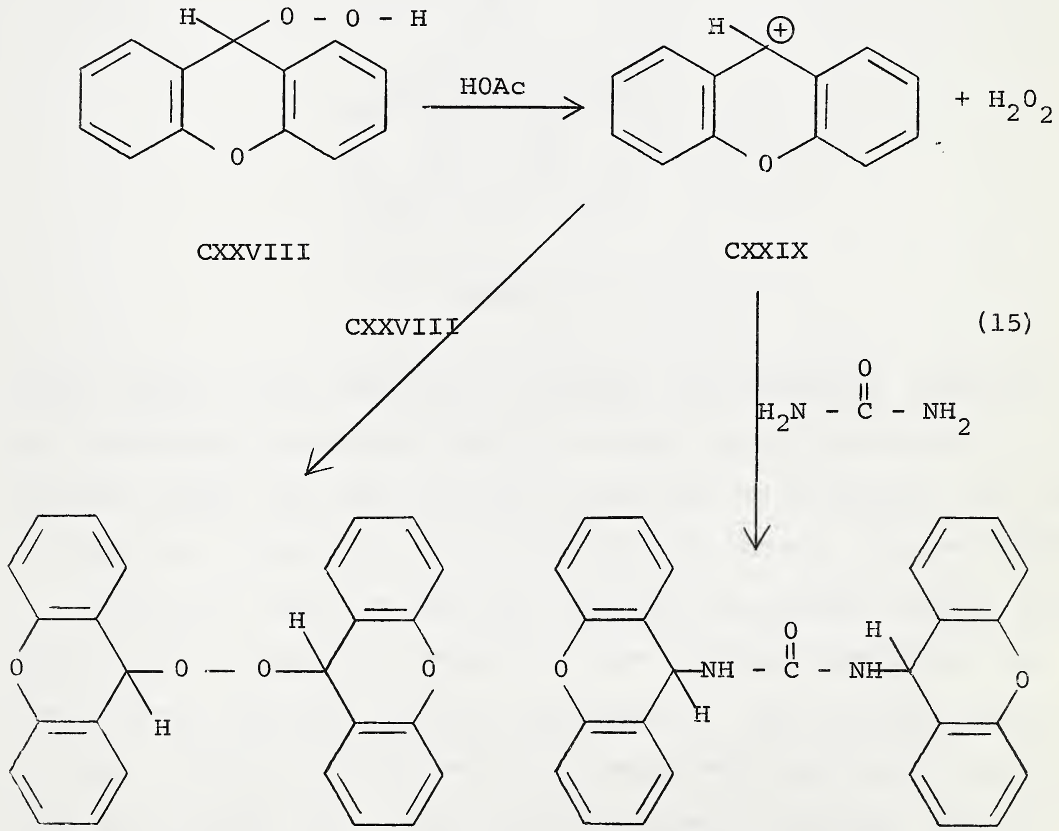
(CXXVII) and hydrogen peroxide. This disproportionation reaction of the hydroperoxide was shown to go through the intermediate carbonium ion (CXXIX). The presence of the latter was demonstrated by its reaction with added nucleophilic reagents. For example, when urea was added they recovered dixanthenyl urea (LXXXVI) (equation 15).



CXXVIII CXXIX

(15)

CXXVII LXXXVI

Similarly, by adding a series of alkyl hydroperoxides (CXXX) in chloroform to a solution of xanthydrol (I) in acetic acid, they recovered the corresponding alkyl xanthenylperoxides (CXXXI) (29).

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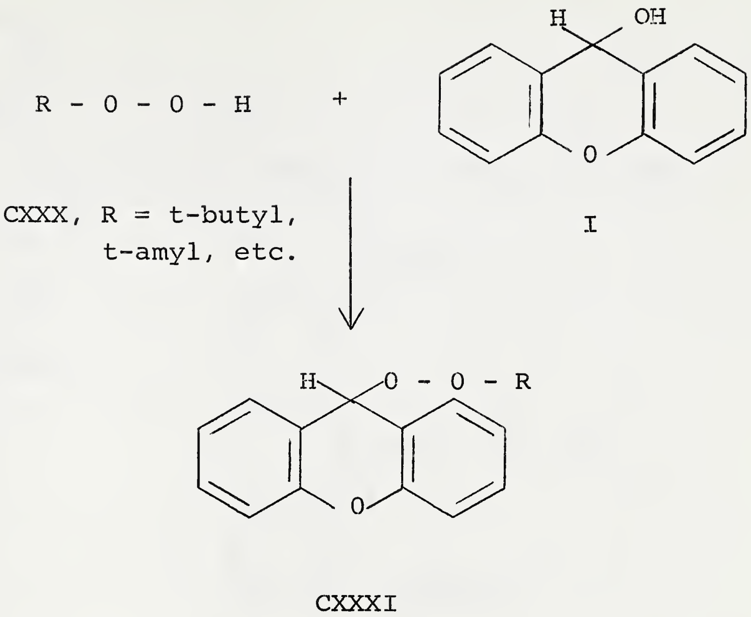
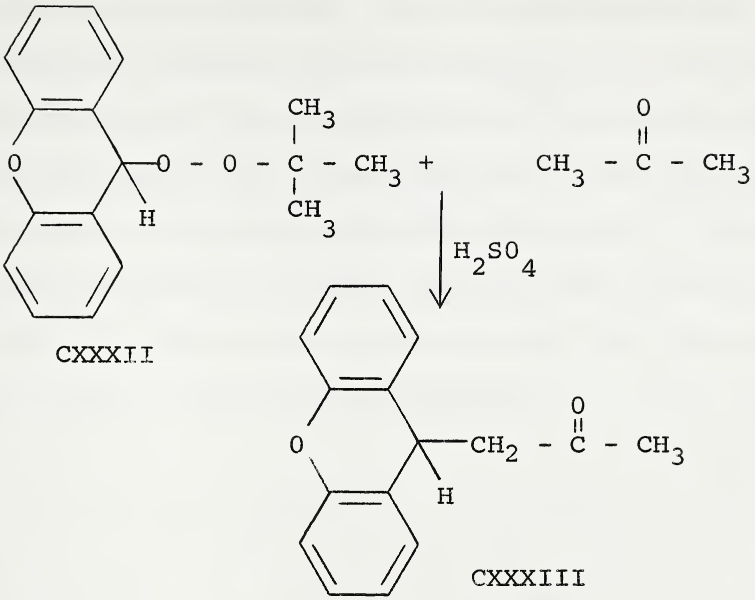


These workers also found that xanthenyl hydroperoxide (CXXVIII) and dixanthenyl peroxide (CXXVII) reacted with s-trialkoxylbenzenes under the same reaction conditions as xanthydrol (I) itself, to yield the identical 1,3,5-trialkoxy-2-xanthenyl benzene (LXIV).

Similarly, many of the alkyl xanthenyl peroxides (CXXXI) condensed with nucleophilic agents to give products identical with those formed from the alcohol (xanthydrol). One of these condensations yielded a product which has resisted preparation directly from the alcohol (I). Thus, t-butylxanthenyl peroxide (CXXXII) when condensed with acetone, in the presence of sulfuric acid, yielded xanthenyl acetone (CXXXIII) (equation 16).



CXXXII

CXXXIII

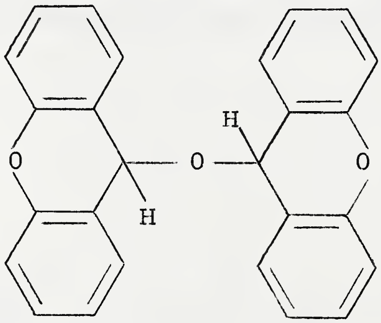
(16)

Pastour (35) had attempted to prepare xanthenylacetone (CXXXIII) by the condensation of acetone and xanthydrol, however, he recovered only the dixanthenylether (CXXXIV). Fosse and Robyn (109) prepared CXXXIII earlier by the hydrolysis of ethylxanthenylacetoacetate.

Bassey et al. (110) have also reported the preparation of two alkyl xanthenyl peroxides by a procedure similar to that of Davies described above (29).

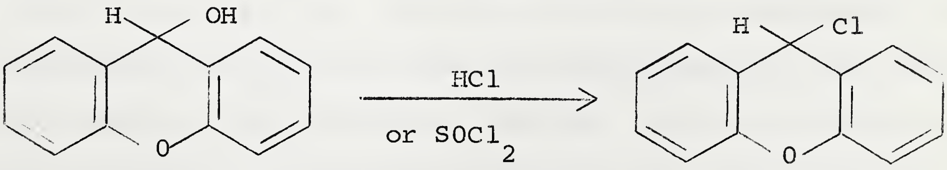
Dixanthenylether (CXXXIV) was first reported by Meyer and Saul (6) as resulting from the heating of xanthydrol in petroleum ether for several days. Kny-Jones and Ward (26) later obtained the ether (CXXXIV) in good yields by simply heating moist xanthydrol (I), freshly prepared from xanthone (IX), at 175°. The ether (CXXXIV) has also been obtained many times as an auxiliary product, or as the exclusive product, in many attempted preparations of other xanthenyl derivatives (35,51,106).

Latif and coworkers (111) have recently shown that dixanthenyl ether(CXXXIV), like the hydroperoxide (CXXVIII) and the peroxide (CXXVII), reacted readily with active hydrogen compounds to give the corresponding 9-xanthenyl derivatives, identical again with those obtained by the acid catalyzed reaction of these compounds with xanthydrol. The reaction proceeds smoothly by simply refluxing the reactants in absolute ethanol, no acetic acid being required, and thus provides a new route to the 9-substituted xanthenes.



CXXXIV

Xanthydrol (I) in dry ether when treated with dry HCl gives xanthydryl chloride (CXXXV) (112). The chloride has also been prepared by the reaction of (I) with thionyl chloride in boiling liquid petroleum (26) or in dry ether (113,114).

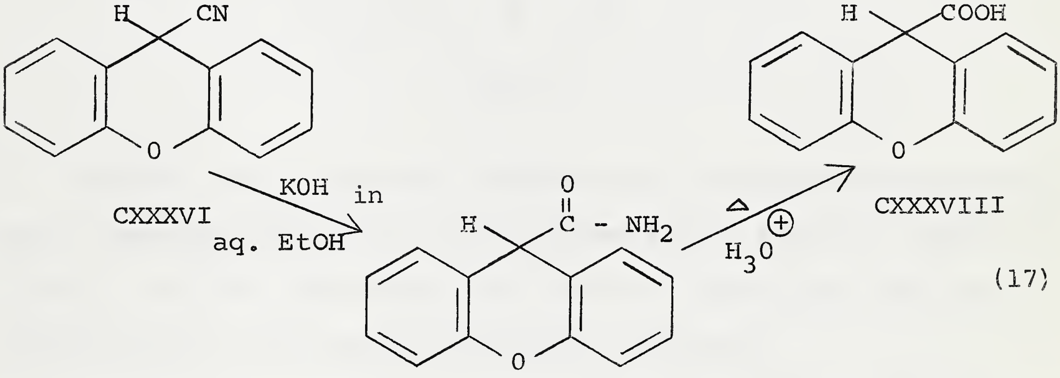


I

CXXXV

Xanthydryl bromide is made in a manner similar to that used for the chloride (112), and the perchlorate is made by treating xanthydrol with 70% perchloric acid (115).

Condensation of xanthydrol (I) with KCN in acetic acid yields 9-xanthenylcarbonitrile (CXXXVI)(39). Kasztreiner and Vargha (113) prepared the above compound by first forming the chloride (CXXXV) with thionyl chloride, and then treating the resulting product with CuCN. They then treated the nitrile (CXXXVI) with KOH in aqueous ethanol to give 9-xanthenyl carboxamide (CXXXVII), which on continued boiling followed by acidification was hydrolyzed to 9-xanthenyl carboxylic acid (CXXXVIII). (equation 17).



CXXXVI

CXXXVIII

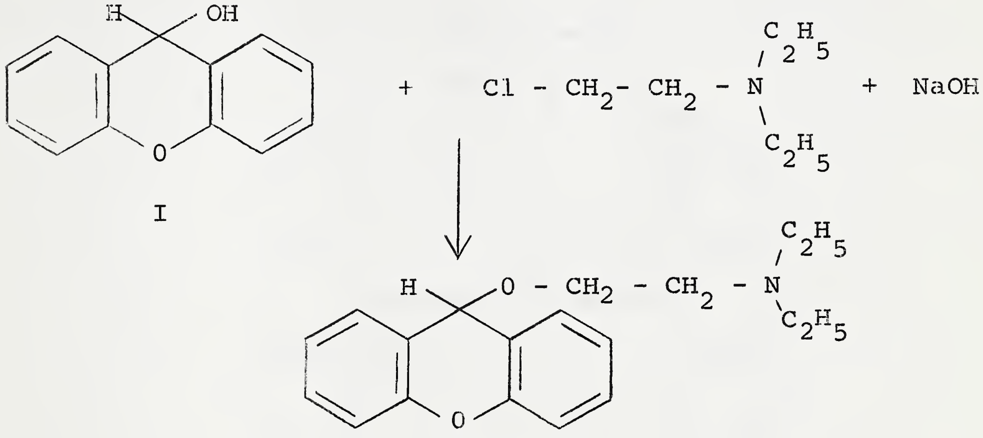
(17)

CXXXVII

Xanthydrol has also been employed as a reagent in color tests for glucosides (116,117) and alkaloids (118).

As mentioned earlier, the main reactions of xanthydrol involve the carbonium ion resulting from the displacement of the entire hydroxyl group in the molecule. However, there are several reports in the literature of reactions involving only the hydroxylic hydrogen atom to yield the corresponding ethers.

Cusic (119,120) prepared 9-xanthenyl 2(diethylamino)-ethyl ether (CXXXIX) by heating xanthydrol (I), diethylaminoethyl chloride, and anhydrous powdered sodium hydroxide at 90-100° for 8 to 10 hours (equation 18).


H
OH
O
+
$Cl - CH_2 - CH_2 - N(C_2H_5)_2$
+
NaOH
H
$O - CH_2 - CH_2 - N(C_2H_5)_2$
O

I

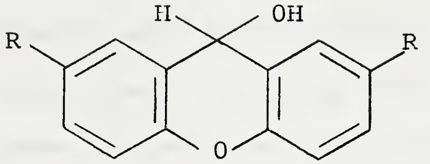
(18)

CXXXIX

Riffkin and Rubin (121) prepared the corresponding dimethylamino compound employing a fine dispersion of metallic sodium in xylene, to form the sodium derivative of xanthydrol and then treating this with dimethylaminoethyl chloride.

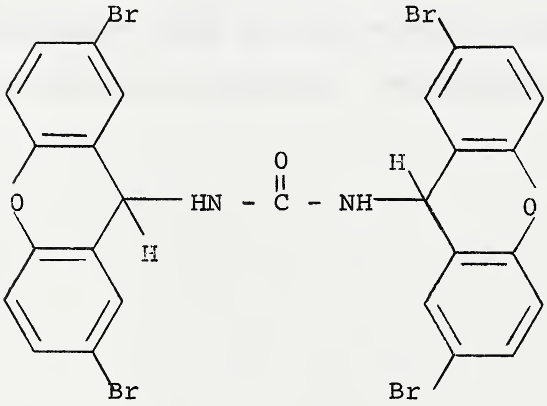
Several halogenated xanthydrols have been prepared and studied as reagents for nucleophilic agents.

2,7-Dibromo (CXL) (122) and 2,7-diiodoxanthydrol (CXLI) (123) have been synthesized and reacted with a number of active hydrogen compounds. Bertrand (124) prepared N,N^1-bis(2,7-dibromo-9-xanthenyl) urea (CXLII), however, unlike the comparable product with xanthydrol, the reaction was not quantitative. He also prepared several derivatives of (CXL) with barbiturates.



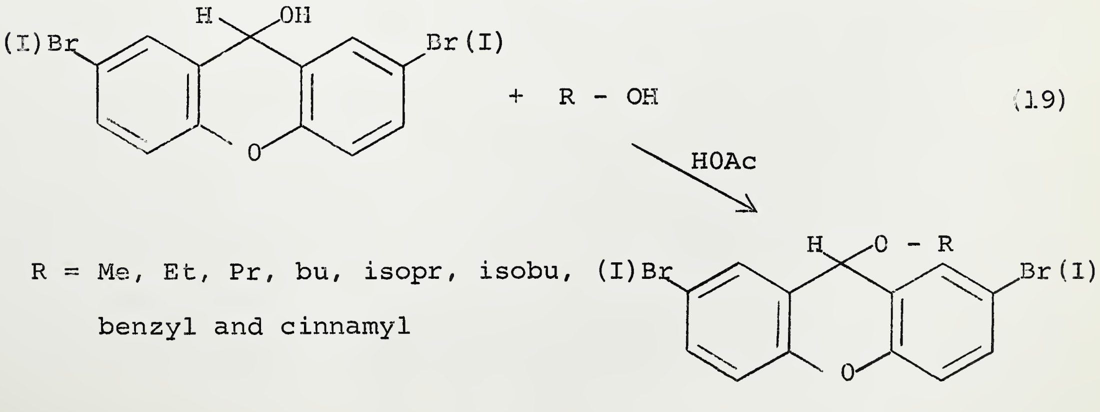
CXL, R = Br

CXLI, R = I



CXLII

One very significant difference in reactivities has resulted from the studies with the halogenated xanthydrols. Unlike xanthydrol (I), the 2,7-dibromo (CXL) and 2,7-diodo (CXLI) derivatives react with alcohols to give the corresponding crystalline ethers (CXLIII)(125,126)(equation 19).



(19)

R = Me, Et, Pr, bu, isopr, isobu, benzyl and cinnamyl

CXLIII

[illegible]

[illegible]

[illegible]

[illegible]

Primary and secondary alcohols reacted readily; however, the tertiary alcohols failed to react.

Finally, Tsukamoto and Ijichi prepared 2- and 3-chloroxanthydrol and studied their reactions with a series of amides. They found that these did not react as readily as xanthydrol itself, but that the derivatives were more stable and hence useful as qualitative reagents for the amides.

To our knowledge, this is the extent of the recorded information on reactions involving xanthydrol.

RESULTS AND DISCUSSION

1. The Preparation of 9-Xanthenyl Derivatives of N'-Monosubstituted Sulfanilamides.

The success of Phillips and Frank (1) in the synthesis of N-xanthenylalkylbenzene sulfonamides, by simple condensation of the reactants in glacial acetic acid, prompted an attempt along similar lines. This method, although proving generally successful, yielded products which were highly contaminated with unreacted sulfonamide because of the limited solubility of the latter in this medium. Heating the reaction mixture did not help in overcoming this difficulty. Crude yields in excess of theoretical were not uncommon, and the melting points of the resulting xanthenyl derivatives exhibited wide ranges.

Sawicki and Oliverio (5), obviously experiencing similar solubility problems, employed a sufficiently large volume of acetic acid to completely dissolve the sulfonamide prior to its addition to the xanthydrol solution. In this manner, they recovered their products after allowing the mixture to stand for "over a week". In an attempt to duplicate this procedure, it was found that a very large volume of acetic acid was required in the reaction and that a period of three weeks had elapsed before any product had separated. Since our interests lie in developing a procedure suitable for routine use, in the qualitative identification of sulfonamides, we did not feel justified in pursuing this method further. The exceedingly long reaction time concomitant with the large volume of solvent required would obviously render such a procedure impractical for this purpose.

The nature of the reaction in the presence of a second suitable non-reactive solvent, which was used to completely dissolve the sulfonamide, was then investigated. Ethanol, acetone and dioxane each worked well in this capacity with those sulfonamides demonstrating a high degree of solubility in these media. None of the aforementioned solvents, however, possessed sufficiently broad solubilizing properties to allow it to be employed in a general procedure. N,N-Dimethylformamide (DMF), on the other hand, enjoyed this latter attribute and yielded excellent results. Simple admixtion of a solution of the sulfonamide in DMF with xanthydrol in glacial acetic acid, at room temperature, afforded the corresponding xanthenyl derivatives in good yields. As opposed to the products obtained from glacial acetic acid alone, these derivatives displayed relatively sharp melting points. Separation of the product normally occurred in a matter of seconds, although several sulfonamides required periods ranging up to several hours for complete reaction. This method was rapid, efficient and readily reproducible and accordingly was adopted as the general procedure for the preparation of the 9-xanthenyl derivatives.

2. Purification of the Crude 9-Xanthenyl Derivatives.

What is normally a rather routine procedure, in this instance, presented by far the greatest problems encountered during the course of this project. Consequently, the bulk of the time devoted to this problem was consumed in obtaining analytically pure samples.

The crude derivatives, the majority of which were white in color, on attempted crystallization in the usual manner yielded

yellow products accompanied by a drop and/or an extended range in their melting points. Solvent type did not appear to be a factor as this decomposition was evident in every solvent tested. Of the more than 40 different solvents studied, only about a quarter of these were suitable and in each of these the characteristic signs of decomposition were apparent. Temperature did not appear to be a determining factor, since the results were equally deleterious in solvents ranging in boiling points from approximately 55 to 135°C. None of the solvents studied possessed the required solubility properties of a recrystallizing solvent to render it generally applicable. Dioxane, however, demonstrated the widest utility and consequently the bulk of our efforts at resolving this problem were directed toward this solvent.

Following a suggestion that the peroxide impurities normally present in dioxane may be causing our problems (127), these were removed and subsequent recrystallizations from this solvent were carried out using the peroxide-free dioxane. A definite improvement was observed over a comparable experiment from the unpurified solvent. However, repeated crystallization failed to furnish a pure product, and in fact the degree of decomposition increased with each successive attempt. Our attention was then directed to an apparatus designed after Ulich (128) by means of which the recrystallization was carried out, up to the drying step, in the absence of air. Nitrogen gas was used to flush out the apparatus, and a nitrogen atmosphere was maintained throughout the process. Unfortunately, this precaution offered little if any amelioration over an analogous crystallization, performed in the usual manner, from peroxide-free dioxane.

The decrease in the incidence of decomposition following removal of the peroxides from dioxane suggested the likelihood of an oxidative process operating in the solution. This argument, however, could not be extended to include other equally detrimental solvents which were peroxide-free but yet yielded a similar decomposition. Nevertheless, it was decided to investigate the possibility that this breakdown, be it due to oxidation or some other cause, may be enhanced at elevated temperatures. Accordingly, the recrystallization from peroxide-free dioxane was repeated, at room temperature, using water to effect the crystallization. As an added precaution, nitrogen gas was bubbled through the solution during the process and then entrapped in the flask while allowing for slow crystallization. Although dioxane proved to be far from ideal in this capacity, because of the limited solubilities of the derivatives in the cold solvent, none the less the results were encouraging. The characteristic yellow color previously acquired by the solution was no longer evident and a white product was recovered.

Earlier in our investigation it had been noted that most of the derivatives were extremely soluble in cold pyridine, and thus attempts were made to confirm our observations by resorting to this solvent. Hence, the aforementioned procedure was duplicated with one of these derivatives by employing pyridine rather than dioxane. Our suspicions were confirmed, as it was possible to recrystallize the derivative to a constant melting point with no apparent decomposition. The majority of the other derivatives were also effectively purified by this method. In several instances n-hexane was employed as the miscible solvent, as it

proved to be more efficient than water in effecting crystallization. Several other derivatives, however, could not be adequately purified from either solvent mixture. At first these recrystallizations were performed under nitrogen, but it was later found that this precaution was not essential when using pyridine.

At this point yet another problem arose. Upon drying the purified samples in a drying pistol at 78° (ethanol), effects ranging from a slight change in color and a slight drop in the melting point to quite drastic changes in both these properties were encountered. Thus, it became apparent that the effect of heat was not confined to the derivatives when present in solution, but that it was equally deleterious to these samples when in the dry state. A somewhat similar finding has been reported previously by Phillips and Frank (1). They observed a lowering in the melting points of from 2 to 8° when drying samples of N-xanthenylalkylbenzene sulfonamides at a temperature of 80°. However, no mention was made of any color change. Similarly, they made no reference to any difficulties encountered during purification, for which purpose they employed dioxane (commercial)-water mixtures at the boiling point.

Decreasing the drying temperature to 55° (acetone) yielded similar results. Prolonged drying under vacuo at room temperature (5 days) failed to remove all of the pyridine. Forcing dry nitrogen through the sample, by means of an apparatus designed to remove solvents from compounds affected by heat (129), proved ineffectual. This method is purported to be effective in drying most samples in from 5 to 15 minutes. However, the odor of pyridine was still discernible even after a drying period of an

hour. Having failed in this approach, repeated washings of the sample with n-pentane were resorted to. It was found that providing the washing step was carried out a sufficient number of times, usually about 20 washings of 20 ml. each, a stage was reached when the pyridine odor was no longer perceptible in the sample. As an added precaution, these samples were then dried in a drying pistol at room temperature for a period of 48 hours. Samples prepared in this manner yielded satisfactory elemental analyses.

With those derivatives for which pyridine proved unsuitable, recrystallizations from other solvent systems, also at room temperature, were attempted. Acetone-n-hexane gave good results with certain derivatives, however, with others this same solvent system yielded the characteristic yellow decomposition products. For two of the derivatives, recrystallization from two different solvent pairs was necessary before a pure product could be obtained.

It would appear, therefore, that although heat is undoubtedly the major cause of this decomposition, it may not be the sole determining factor. Likewise, peroxides, though obviously contributing to this problem, cannot be linked entirely to the breakdown of these compounds. Thus, a combination of factors, some of which are not known to us, must be responsible for the instability of these derivatives.

An attempt was made to isolate this decomposition product by means of thin layer chromatography. A comparison of the chromatograms from the crude white product with that of the yellow recrystallized product, however, failed to reveal an unique spot which could be attributed to the decomposition product. In view

of this result, and since elucidation of its structure was not our aim, further work in this direction was discontinued.

Of the 17 N^1-monosubstituted sulfanilamides for which analytically pure samples of their derivatives were obtained, 7 of these did not correspond to the calculated analyses for mono-xanthenyl derivatives. Recalculation revealed, however, that they agreed in each instance with the values expected for dixanthenyl derivatives. This was a completely unexpected occurrence and therefore created a new structural problem. Sulfanilamide itself, as expected, also afforded a dixanthenyl derivative. As these dixanthenyl derivatives had been obtained from a mole-to-mole reaction with xanthydrol, the preparation of all the derivatives was repeated employing a 2-to-1 molar ratio of xanthydrol to sulfonamide. In each instance, the products obtained from this reaction were identical with those previously obtained from a mole-to-mole ratio. A similar result has been reported by Illari (54) for the reaction of xanthydrol with pyrrole. He found that pyrrole yielded 2,5-dixanthenyl-pyrrole with as little as 0.5 moles or with as much as 4 moles of xanthydrol per mole of pyrrole. Thus, each of the sulfanilamides studied yielded only one product, either the mono or disubstituted, regardless of whether a deficiency or an excess of xanthydrol was employed.

Tables I and II show the recrystallizing solvents, melting points and elemental analyses for the mono- and dixanthenyl derivatives respectively.

TABLE I

Monoxanthenyl-N^1-Monosubstituted Sulfanilamides

Ref. No.	Sulfanilamide, N^1-	Generic Name	Melting Point °C	Recrystallizing solvent [a]	Formula	Analysis Calculated C	Calculated H	Calculated N	Found C	Found H	Found N
1	Acetyl	Sulfacetamide	208-208.5	Pyridine-water	$C_{21}H_{18}N_2O_4S$	63.94	4.60	7.10	63.68	4.82	7.40
2	2-Pyrimidyl	Sulfadiazine	229-229.5[b]	Pyridine-water	$C_{23}H_{18}N_4O_3S$	64.17	4.21	13.02	64.44	4.26	12.75
3	4-Methyl-2-Pyrimidyl	Sulfamerazine	206-207	Pyridine-water	$C_{24}H_{20}N_4O_3S$	64.85	4.54	12.60	64.73	4.60	12.78
4	4,6-Dimethyl-2-pyrimidyl	Sulfamethazine	173.5-174.5	Pyridine-water then Acetone hexane	$C_{25}H_{22}N_4O_3S$	65.48	4.84	12.22	65.55	4.87	12.19
5	2-Pyridyl	Sulfapyridine	220-221[c]	Pyridine-hexane	$C_{24}H_{19}N_3O_3S$	67.11	4.46	9.78	66.96	4.72	9.75
6	2,6-Dimethyl-4-pyrimidyl	Sulfisomidine	180-182	Pyridine-water	$C_{25}H_{22}N_4O_3S$	65.48	4.84	12.22	65.55	4.92	11.95
7	p-Isopropoxybenzoyl	Sulfaproxyline	192-193	Pyridine-water	$C_{29}H_{26}N_2O_5S$	67.68	5.09	5.45	67.71	5.08	5.43
8	Guanyl	Sulfaguanidine	225.5-226.5	Pyridine-hexane	$C_{20}H_{18}N_4O_3S$	60.90	4.60	14.20	60.87	4.62	14.35
9	5-Methyl-3-isoxazolyl	Sulfamethoxazole	205-205.5	Acetone-hexane	$C_{23}H_{19}N_3O_4S$	63.73	4.42	9.69	64.00	4.63	9.60
10	1-Phenyl-5-pyrazolyl	Sulfaphenazole	228-228.5	Acetone-hexane	$C_{28}H_{22}N_4O_3S$	68.00	4.48	11.33	68.19	4.76	11.35

a. All recrystallizations were carried out at room temperature.
b. Literature 246-247 (5)
c. Literature 241-242 (5)

TABLE II

Dixanthenyl-N^1-Monosubstituted Sulfanilamides

						Analysis					
						Calculated			Found		
Ref. No.	Sulfanilamide, N^1-	Generic Name	Melting Point °C	Recrystallizing solvent [a]	Formula	C	H	N	C	H	N
11	--	Sulfanilamide	209-210[b]	Acetone-hexane	$C_{32}H_{24}N_2O_4S$	72.16	4.54	5.26	72.19	4.52	5.06
12	5-Methyl-1,3,4-thiadiazol-2-yl	Sulfamethizole	206.5-207	Chloroform-hexane then Acetone-water	$C_{35}H_{26}N_4O_4S_2$	66.65	4.15	8.88	66.62	4.16	8.90
13	5-Ethyl-1,3,4-thiadiazol-2-yl	Sulfaethylthiadiazole	210.5-211.5	Pyridine-water	$C_{36}H_{28}N_4O_4S_2$	67.06	4.38	8.69	66.96	4.44	8.73
14	2-Thiazolyl	Sulfathiazole	179-180	Pyridine-water	$C_{35}H_{25}N_3O_4S_2$	68.27	4.09	6.83	68.35	4.01	6.54
15	6-Methoxy-3-pyridazinyl	Sulfamethoxypyridazine	223-224	Pyridine-water	$C_{37}H_{28}N_4O_5S$	69.36	4.40	8.74	69.51	4.20	8.66
16	6-Chloro-3-pyridazinyl	Sulfachloropyridazine	184.5-185.5	Pyridine-water	$C_{36}H_{25}ClN_4O_4S$	67.02	3.91	8.68	66.90	4.20	8.82
17	3,4-Dimethyl-5-isoxazolyl	Sulfisoxazole	178.5-179	Acetone-hexane	$C_{37}H_{29}N_3O_5S$	70.79	4.66	6.69	70.33	4.74	6.68
18	2,6-Dimethoxy-4-pyrimidyl	Sulfadimethoxine	194.5-195	Chloroform-hexane	$C_{38}H_{30}N_4O_6S$	68.04	4.51	8.35	62.91[c]	4.32	8.10

a. All recrystallizations were carried out at room temperature.

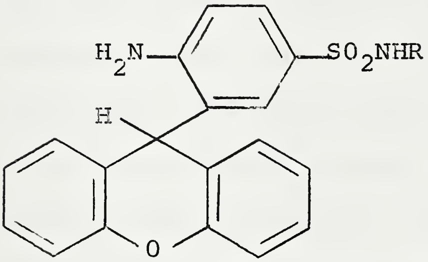
b. Literature 208-209 (1).

c. M.W. Calc. 670.7, Found 680.5.

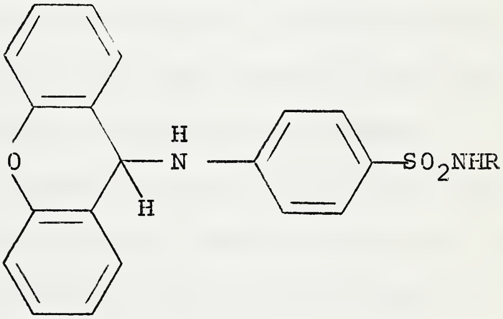
3. Structural Determinations

a). Monoxanthenyl -N^1-Monosubstituted Sulfanilamides.

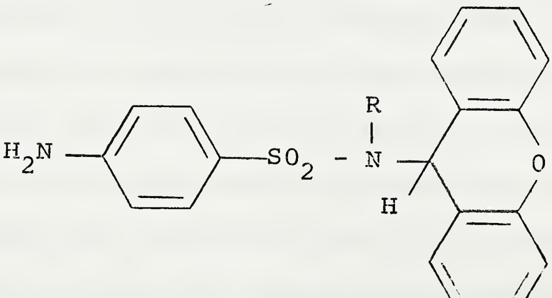
An examination of the molecular composition of the N^1-monosubstituted sulfanilamides suggested three possible sites for electrophilic substitution by xanthydrol, as exemplified by structures CXLIV, CXLV, and CXLVI respectively.



CXLIV



CXLV



CXLVI

As mentioned in the statement of the problem, published evidence is available in support of structure CXLIV (1) as well as in support of CXLV (5). Structure CXLVI, although possible, is the least likely because of the lack of reactivity exhibited

[illegible]

[illegible]

by several secondary sulfonamides and secondary carboxyamides which have been studied previously (1,2,3,4).

Since no direct evidence was available to either uphold or belittle this latter structure, this appeared to be a good place to begin our investigation. Assuming structure CXLVI to be the correct one, its distinguishing feature is the fact that it is a tertiary sulfonamide as opposed to the other two possibilities both of which are secondary. Solubility tests revealed that all the derivatives listed in Table I, with the exception of that of sulfaphenazole (Ref. No. 10), were insoluble in 5% sodium hydroxide. The above compound showed a slight solubility indicated by the presence of a light turbidity upon neutralization of the alkaline test solution. This apparent lack of solubility in dilute alkali constituted strong evidence in favor of structure CXLVI. In an attempt to substantiate this result, the derivatives were titrated nonaqueously, as acids, in N,N-dimethylformamide (DMF). Contrary to the above evidence each of the compounds in Table I, with the exception of the derivative of sulfaguanidine (Ref. No. 8), proved to be titratable. The failure of this derivative to titrate was not disturbing, since sulfaguanidine itself cannot be titrated as an acid by this method (130). This is due to its very weakly acidic nature,too weak in fact to be measured in aqueous solution (131). The titration data are presented in Table III.

TABLE III

Nonaqueous Titration of Monoxanthenyl-N^1-Monosubstituted Sulfanilamides as Acids.

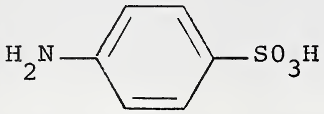
Ref. No.	Derivative of	Molecular Weight	
		Calculated	Found
1	Sulfacetamide	394.4	393.7
2	Sulfadiazine	430.5	437.8
3	Sulfamerazine	444.5	449.2
4	Sulfamethazine	458.5	489.5[a]
5	Sulfapyridine	429.5	431.6
6	Sulfisomidine	458.6	459.3
7	Sulfaproxyline	514.6	520.9
8	Sulfaguanidine	Not Titratable	
9	Sulfamethoxazole	433.5	437.7
10	Sulfaphenazole	494.6	521.8

a. on a partially purified sample.

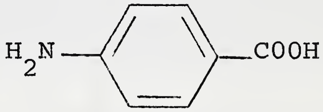
Therefore, although we are at a loss to explain the apparent insolubility in dilute alkali of these derivatives, their titratability rules out substitution of the amide hydrogen and hence excludes structure CXLVI as a possibility.

To distinguish between CXLIV and CXLV, a model compound was sought which would serve to represent the structural features of the N^1-monosubstituted sulfanilamide molecule essential for chemical reactivity with xanthydrol. Accordingly, it had been hoped to employ sulfanilic acid (CXLVII) as this model. However, the compound was found to be quite insoluble in N,N-dimethylformamide, as well as in glacial acetic acid, and therefore its behavior

with xanthydrol could not be determined under identical reaction conditions. No attempt was made at finding suitable reaction conditions for it. In lieu of the above compound, p-aminobenzoic acid (CXLVIII) was selected as the model.



CXLVII



CXLVIII

Treatment of CXLVIII with xanthydrol, by the general procedure, yielded a crystalline derivative which was also difficult to purify. Elemental analysis corresponded to that calculated for a monoxanthenyl derivative. Benzoic acid under the same reaction conditions failed to react. Acetylation of the amino group likewise destroyed activity. These facts served to illustrate the involvement of the amino group in the condensations with xanthydrol. Furthermore, the failure of p-acetamidobenzoic acid to react verified previous similar findings and added support to the exclusion of CXLVI from the probable structures.

Blocking the two ortho positions in p-aminobenzoic acid by bromination, to give 3,5-dibromo-p-aminobenzoic acid (CXLIX), and subsequent treatment with xanthydrol (I) once again afforded no reaction. Only the starting material CXLIX was recovered, as was evident from its infrared spectrum (Figure 1), which was identical with that of the pure compound (Figure 2).

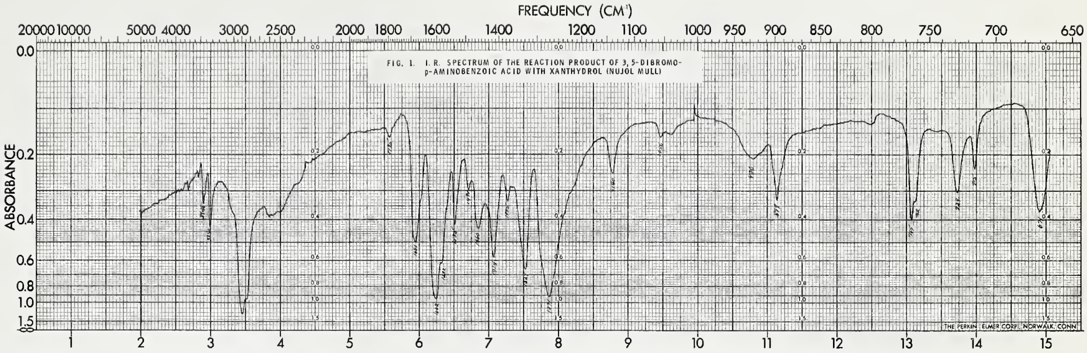


FIG. 1. I.R. SPECTRUM OF THE REACTION PRODUCT OF 3,5-DIBROMO-p-AMINOBENZOIC ACID WITH XANTHYDROL (NUJOL MULL)

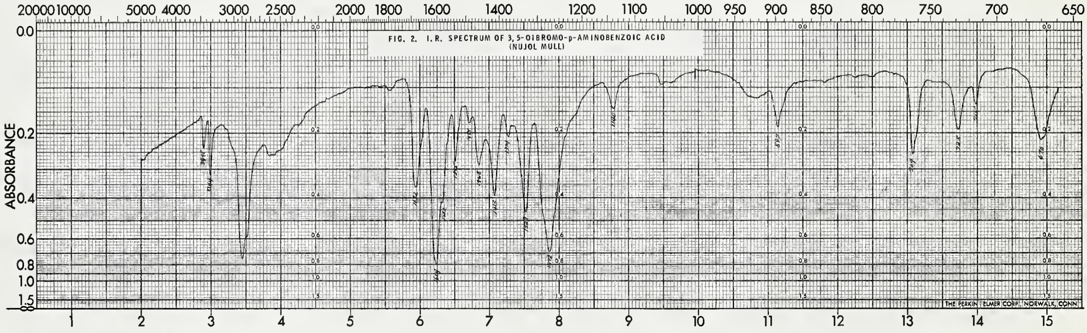


FIG. 2. I.R. SPECTRUM OF 3,5-DIBROMO-p-AMINOBENZOIC ACID (NUJOL MULL)

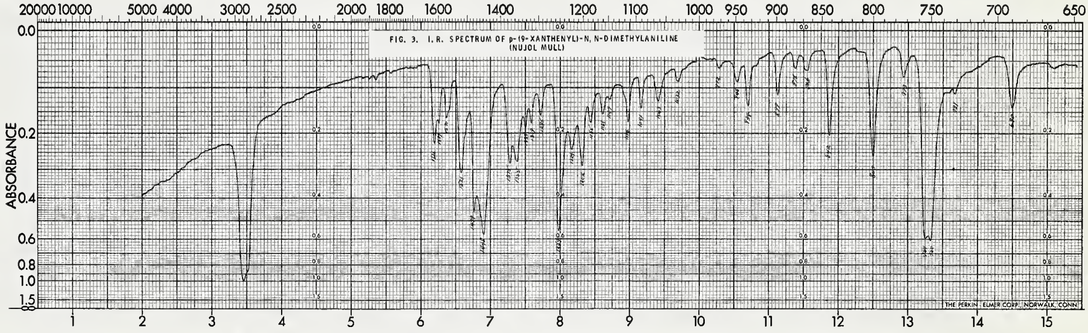


FIG. 3. I.R. SPECTRUM OF p-(9-XANTHENYL)-N,N-DIMETHYLANILINE (NUJOL MULL)

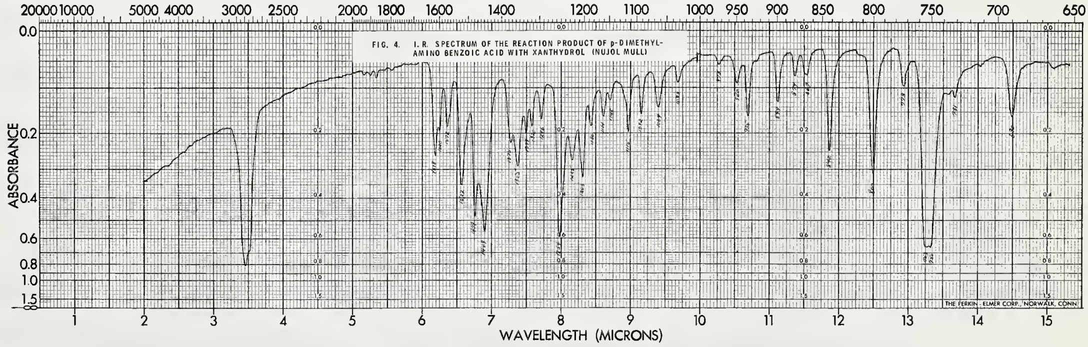
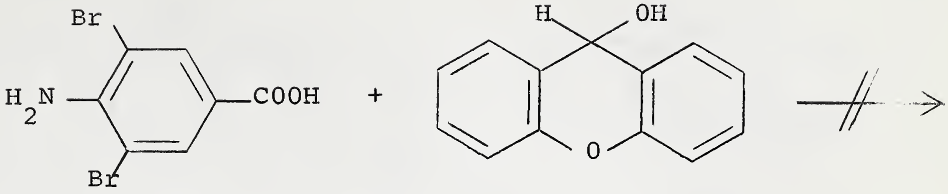


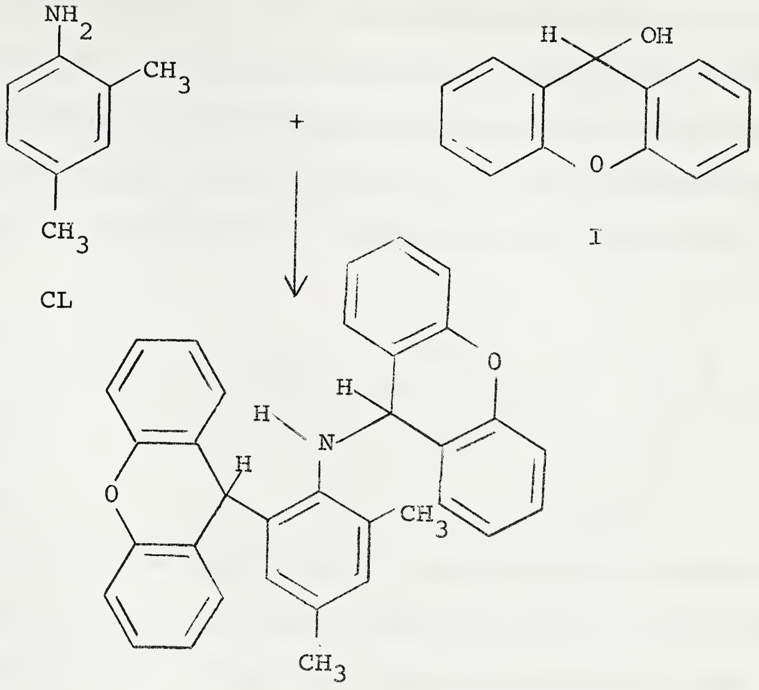
FIG. 4. I.R. SPECTRUM OF THE REACTION PRODUCT OF p-DIMETHYLAMINO BENZOIC ACID WITH XANTHYDROL (NUJOL MULL)



CXLIX I

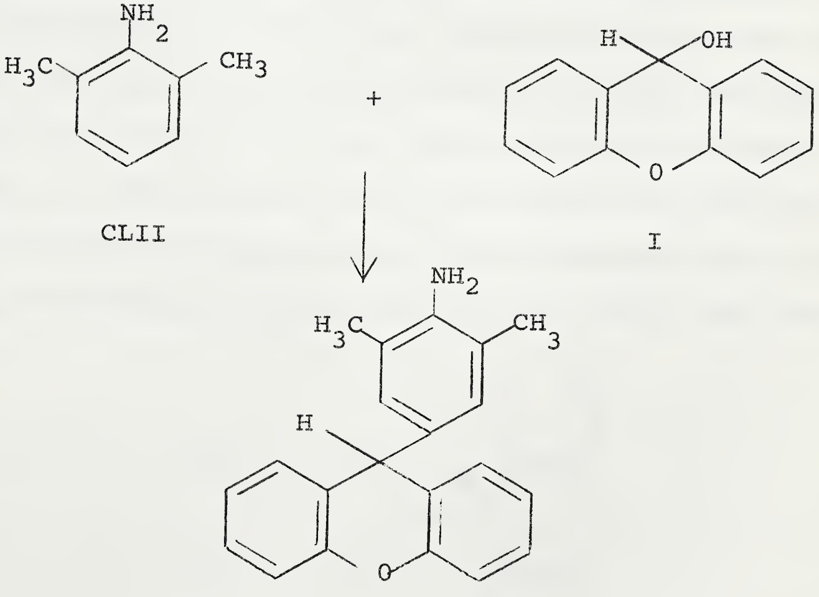
Failure of the above compound to react with xanthydrol would tend to support substitution in the now occupied ortho position as indicated in structure CXLIV. However, the possibility exists that the two relatively large bromine atoms could be sterically hindering substitution of the amino hydrogen and thus account for this observation. This latter supposition is supported by some results reported by Adriani (3). He found that when 2,4-xylidine (CL) was condensed with xanthydrol (I) it yielded the dixanthenyl derivative CLI (equation 20). 2,6-Xylidine (CLII), on the other hand, gave only the ring subsituted monoxanthenyl derivative CLIII (equation 21). Since the van der Waals' radii of a bromine atom and a methyl group are very nearly identical (approximately 2.0 Å (132)), the steric effects of these groups would also be expected to be similar. Thus, the result obtained with the dibromo compound CXLIX could be interpreted to favor either ring (CXLIV) or nitrogen (CXLV) substitution.

Having failed to gain any conclusive evidence by this means, another approach was embarked upon. If substitution was occurring with an amino hydrogen, then replacement of the hydrogens by methyl groups should result in a lack of reactivity. If, on the


NH2
CH3
CH3
CL
H
OH
O
I
+
O
H
H
N
H
O
CH3
CH3

(20)

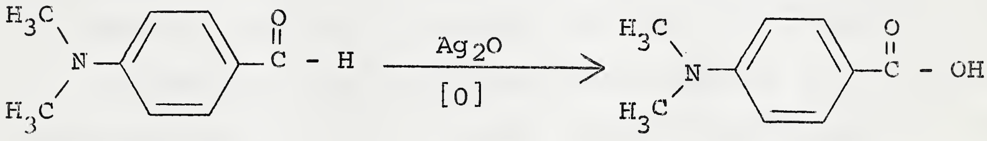
CLI


NH2
H3C
CH3
CLII
+
H
OH
O
I
NH2
H3C
CH3
H
O

(21)

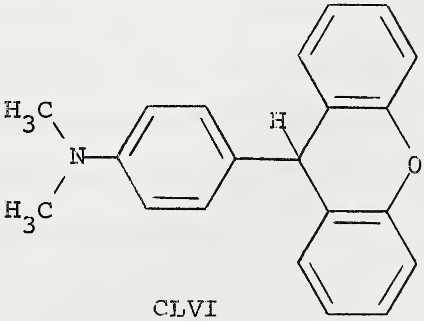
CLIII

other hand, substitution was occurring on the ring, then as the dimethylamino group is even more strongly ring activating, the corresponding o-substituted xanthenyl derivative should result. Accordingly, p-dimethylaminobenzoic acid (CLIV) was prepared by the silver oxide oxidation of the corresponding aldehyde (CLV) and subsequently condensed with xanthydrol.



CLV CLIV

After 18 hours at room temperature, a white product separated which, unlike all previous derivatives, was easily purified by recrystallization from ethanol in the normal manner. The elemental analysis, however, did not correspond to that calculated for the monoxanthenyl derivative. Upon calculating the empirical formula to fit the analysis, the formula $C_{21}H_{19}NO$ was obtained, which was a molecule of CO_2 less than the expected formula of $C_{22}H_{19}NO_3$. It thus appeared that decarboxylation had occurred, and that a xanthenyl derivative of N,N-dimethylaniline, most likely p-(9-xanthenyl)-N,N-dimethylaniline (CLVI), was recovered.



CLVI

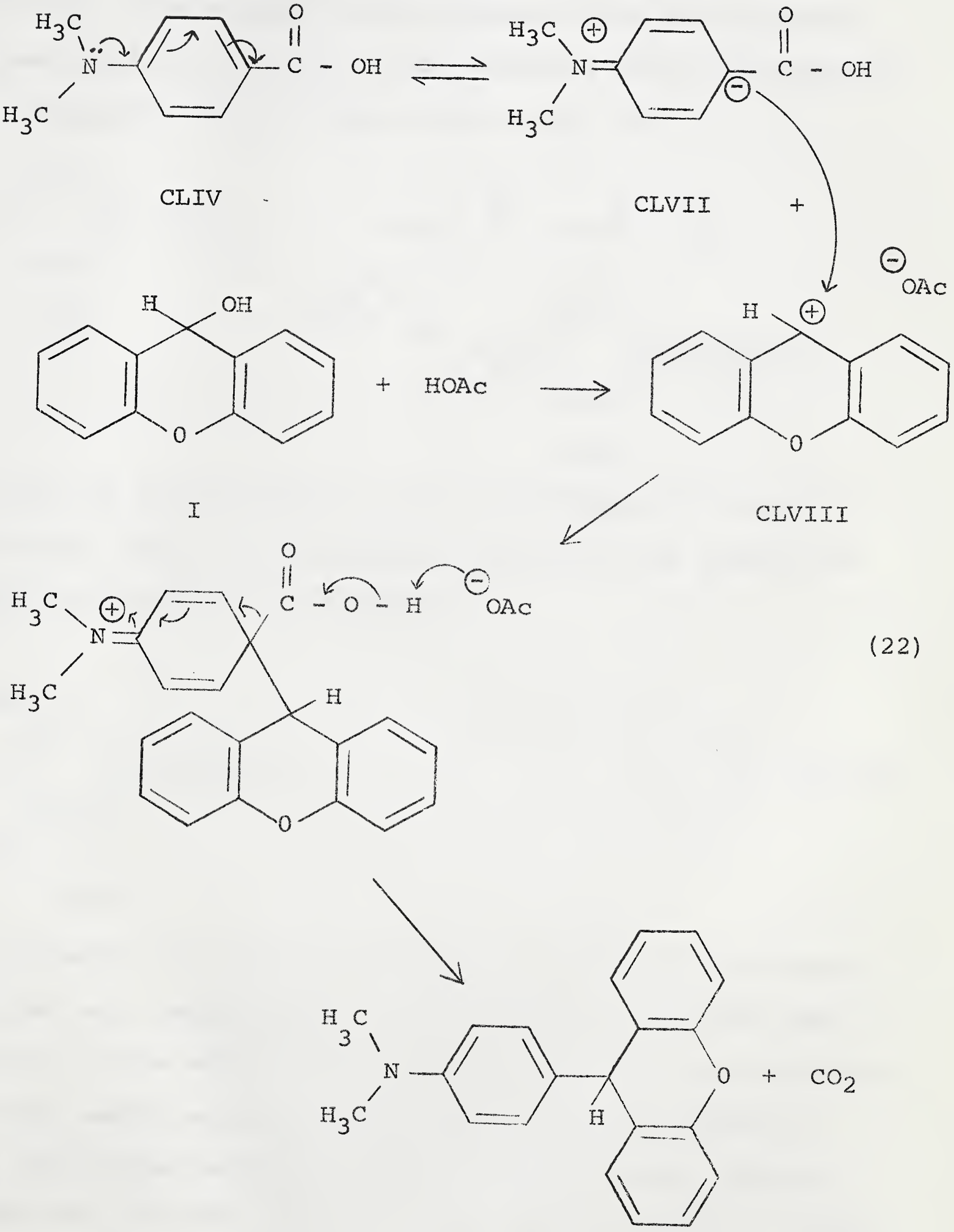
[illegible]

[illegible]

The identity of the product was confirmed by synthesizing this compound from N,N-dimethylaniline and xanthydrol, as reported previously (3, 53, 133). The product from the above reaction showed an identical melting point, and gave no depression from a mixed melting point with the compound isolated from the reaction of xanthydrol with the acid (CLIV). The infrared spectra of these two products were also identical (see Figures 3 and 4).

A plausible mechanism which would explain the reaction sequence leading to this product (CLVI), would appear to be that illustrated in equation 22. Due to the electron donating character of the p-dimethylamino group, an important contributor to the resonance forms of p-dimethylaminobenzoic acid (CLIV) is the zwitterion CLVII. An attack by this species (CLVII) on the xanthylium ion (CLVIII), followed by decarboxylation would then yield the recovered product (CLVI). Burmistrov (134) found that p-dimethylaminobenzoic acid underwent azo coupling by a displacement of CO_2. A similar displacement was reported with other para substituents, including the 9-xanthenyl radical.

This result suggests that p-substituted dimethylanilines will not undergo ortho substitution with xanthydrol, probably due to the influence of steric factors. Thus, a negative result with the above reaction could not have been correctly interpreted as support for substitution on the nitrogen. Corroboration of this postulate came from the fact that p-dimethylaminobenzaldehyde, when condensed with xanthydrol, yielded no reaction.


H3C
N
O
C - OH
H3C
+
H
OH
O
+ HOAc
OAc
H
O
C - O - H
OAc
N
H
N
O
+ CO2
H

CLIV

CLVII

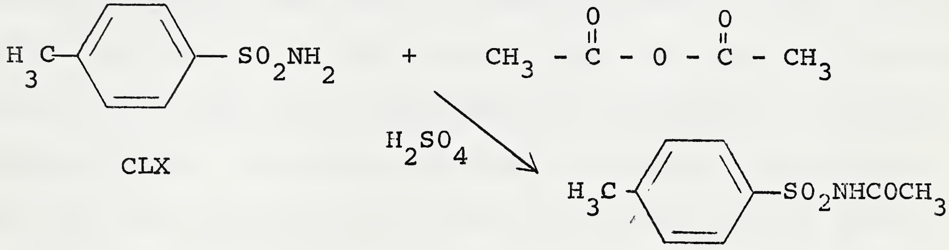
I

CLVIII

CLVI

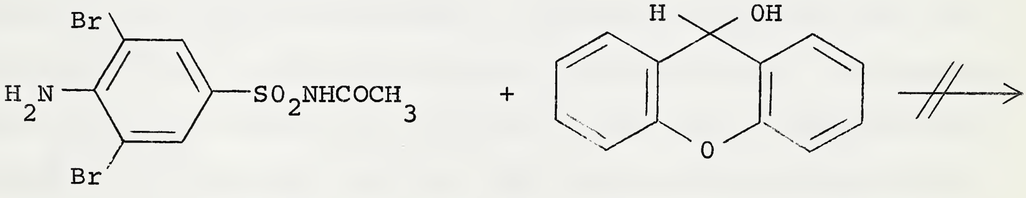
(22)

Some similar studies involving alterations of the sulfacetamide molecule substantiated our findings with p-aminobenzoic acid, but added no new light to the question of the site of substitution. Replacement of the p-amino group with a methyl group destroyed the activity. This compound (CLIX) was prepared by the acetylation of p-toluene sulfonamide (CLX).



CLIX

Bromination of sulfacetamide to give 3,5-dibromo-N^1-acetylsulfanilamide (CLXI), and subsequent treatment with xanthydrol (I), likewise resulted in no reaction.



CLXI I

Several attempts at preparing Schiff's bases of some xanthenyl derivatives with benzaldehyde, in an effort to establish the presence of a free amino group, yielded only starting materials. This result would tend to indicate that the amino group is already substituted and thus favors structure CXLV. However, this does not rule out structure CXLIV, since it could be argued that the bulky xanthenyl group in the ortho position was sterically

hindering the formation of the Schiff's base.

Attempts at distinguishing structure CXLIV from CXLV by N.M.R. spectroscopy proved unsuccessful. The main difficulty encountered concerned the insolubilities of the derivatives in the common N.M.R. solvents, such as carbon tetrachloride and deuterochloroform. It was therefore necessary to resort to less suitable solvents such as N,N-dimethylformamide (DMF) and pyridine. The N.M.R. spectrum of sulfacetamide in DMF and that of its 9-xanthenyl derivative in DMF, and in pyridine, are reproduced in Figures 5,6 and 7 respectively. Not being the ideal solvents, coupled with the fact that the protons of an amino group are normally hard to isolate, even under ideal conditions (135), no satisfactory interpretation of these spectra could be made. Further attempts with this physical tool were therefore discontinued.

Color tests, specific for primary aromatic amines, were then investigated. The standard diazotization and coupling test was found to have several drawbacks. Firstly, the derivatives were quite insoluble in the acid solution, and secondly, a relatively large amount of sample was required in the test. Despite their incomplete solubility, however, the results from several tests appeared to be positive. Both of these problems were overcome by employing a modification of the Bratton and Marshall test for free amino groups (136). This test employs the same principle of diazotization and coupling, but requires only a fraction of a milligram of sample. By employing N,N-dimethylformamide (DMF) rather than dilute base to dissolve the samples, the solubility problem was also solved. All of the monoxanthenyl derivatives listed in Table I gave a positive result with this test. This

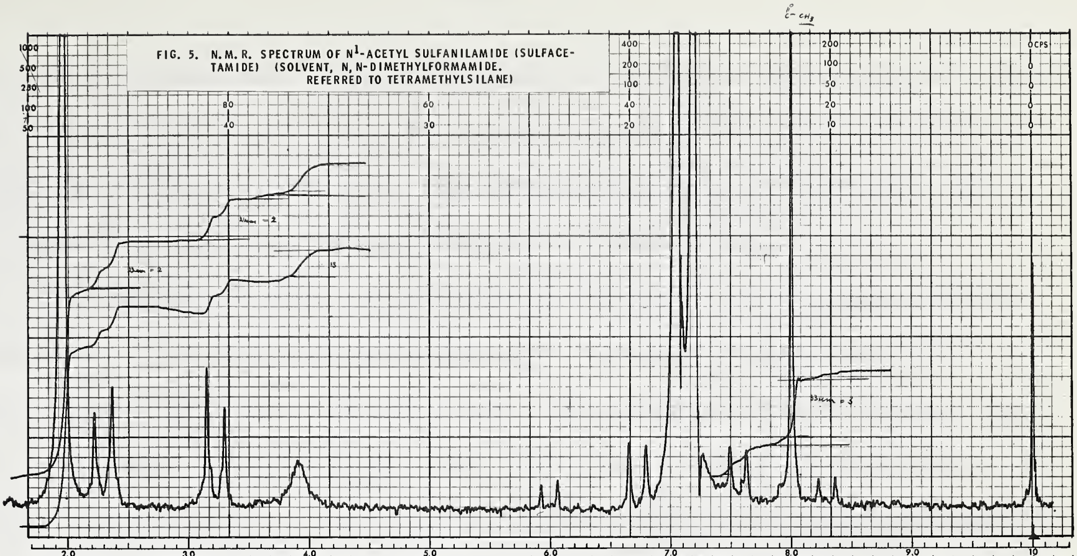


FIG. 5. N.M.R. SPECTRUM OF N^1-ACETYL SULFANILAMIDE (SULFACETAMIDE) (SOLVENT, N,N-DIMETHYLFORMAMIDE. REFERRED TO TETRAMETHYLSILANE)

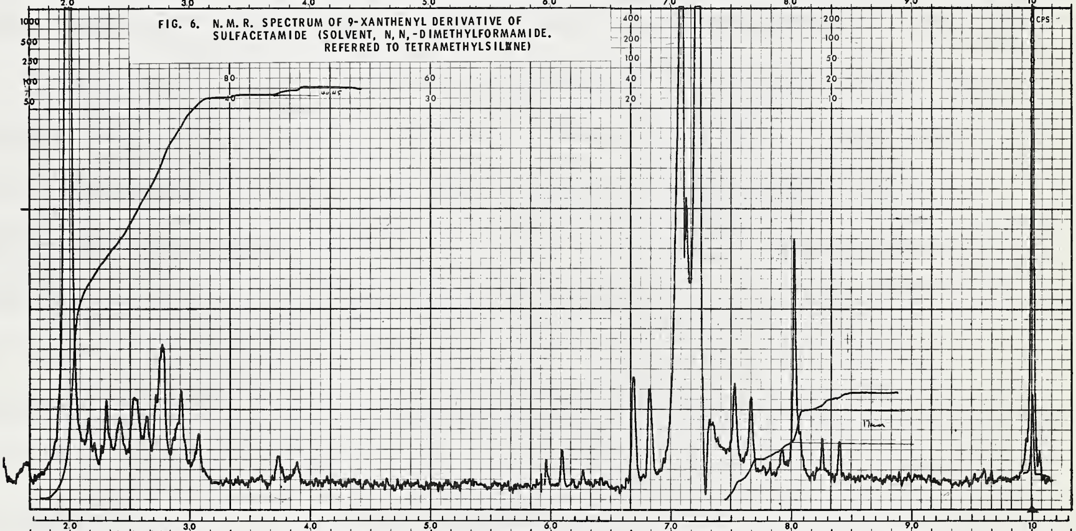


FIG. 6. N.M.R. SPECTRUM OF 9-XANTHENYL DERIVATIVE OF SULFACETAMIDE (SOLVENT, N,N,-DIMETHYLFORMAMIDE. REFERRED TO TETRAMETHYLSILANE)

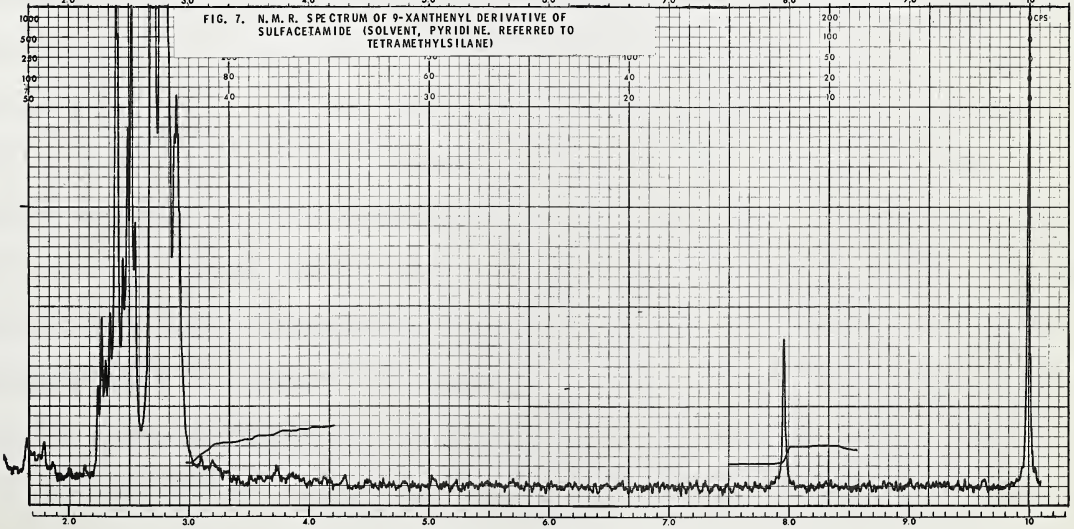


FIG. 7. N.M.R. SPECTRUM OF 9-XANTHENYL DERIVATIVE OF SULFACETAMIDE (SOLVENT, PYRIDINE. REFERRED TO TETRAMETHYLSILANE)

result confirmed the similar finding by Phillips and Frank (1) for the dixanthenyl derivative of sulfanilamide. It thus constituted strong evidence in favor of the ortho substituted structure CXLIV in which the amino group had not been substituted.

The furfural test for free amino groups (137), based on the formation of a colored Schiff's base, however, yielded negative results with these same derivatives. An exception to the above was the derivative of sulfaphenazole (Ref. No. 10) which afforded a weakly positive test. Similarly, the sodium pentacyanoaquoferriate color test for free amino groups (138) furnished negative results.

These conflicting results prompted us to reinvestigate the results obtained by the Bratton and Marshall test. Upon adding 0.5 N hydrochloric acid to a solution of the monoxanthenyl derivative of sulfacetamide, a yellow color developed, and an odor characteristic of xanthydrol was discernible. The acid solution was neutralized with dilute base, and the resulting solution evaporated to dryness on a water bath. A sample of this residue, when tested with furfural, now gave a positive test. It became apparent that the derivatives were being decomposed in acid solution, thus regenerating some of the free amine which accounted for the positive result. Adriani (3) had previously reported the instability of nitrogen substituted xanthenyl derivatives of aromatic amines in concentrated hydrochloric acid. In fact, he utilized the yellow color formed in this acid as a means of distinguishing nitrogen-substituted from ring-substituted derivatives. He attributes this decomposition to the relatively weak nature of the carbon-nitrogen bond in these derivatives, as compared to the carbon-carbon bond in ring-substituted derivatives which are stable in acid. As he

points out, however, a positive result must be interpreted with caution, as other types of nitrogen-substituted derivatives (for example dixanthenyl urea) as well as some carbon-substituted ones (for example the xanthenyl derivative of malonic acid) also give positive tests. The yellow color has been attributed to the formation of an xanthylium ion. It would thus appear that a similar decomposition occurs with the derivatives in the present study even in 0.5 N hydrochloric acid, and that a sufficient amount of the free amine is liberated to yield a positive test with nitrous acid. As the nitrogen-substituted derivatives of amines studied by Adriani presented no problems with purification, it may be concluded that the nitrogen-carbon bond in the xanthenyl derivatives of sulfanilamides must be substantially weaker. This fact would consequently account for the extreme instability of these compounds, and by analogy, the yellow color observed in these decompositions may be attributed to the formation of xanthylium ion.

Confirmation of nitrogen substitution was obtained by an examination of the infrared spectra of the xanthenyl derivatives. The spectra of the parent sulfanilamides showed two bands in the 3500-3300 cm^{-1} region, characteristic of the stretching vibrations of an unsubstituted primary aromatic amine. In addition, they displayed a strong to medium band in the 1640-1590 cm^{-1} region normally attributed to the NH deformation frequency in primary amines. By contrast, the spectra of the xanthenyl derivatives of these sulfanilamides possessed only a single band in the former region, a distinguishing feature for secondary amines.

The effects on the NH deformation absorption, in the transition from primary to secondary amines, is of less diagnostic value. In aliphatic amines, the band intensity is greatly weakened by this transformation to such an extent that it is frequently no longer detectable. With aromatic amines, however, the position is confused to some extent by the presence of C=C stretching absorptions in the same region coupled with the fact that in certain instances these became intensified when a nitrogen atom is attached directly to a ring (139). Consequently, the discriminative utility of the deformation frequency is somewhat limited. Nevertheless, the character of this band in the derivatives added support to the evidence derived from the stretching vibrations. The 1640 cm^{-1} band was completely absent in five of the compounds, greatly weakened in intensity in one instance, and slightly diminished in the remainder. The band positions described above are summarized in Table IV, and the original spectra are reproduced in Figures 8 to 27 inclusive. As mentioned earlier, Sawicki and Oliverio (5) had previously assigned the N^4-xanthenyl structure to the derivatives of sulfadiazine and sulfapyridine on the basis of a single band at 2.9 microns.

As was true with the results from the color tests, the derivatives of sulfaguanidine and sulfaphenazole were exceptions with regard to infrared evidence. The spectra in both instances still showed two bands in the NH stretching region and the deformation band remained relatively unaltered. In the case of sulfaguanidine (CLXII) the appearance of two bands in the former region may be due to the presence of other NH_2 groups in the molecule, thus complicating the interpretation of this spectrum. However,

TABLE IV

N-H Stretching and Bending Vibrations of Parent N^1-Monosubstituted Sulfanilamides and their N^4-Xanthenyl Derivatives

Ref. No.	COMPOUND	ν NH (cm^{-1})		δ NH_2 (cm^{-1})		Fig. No.
		Parent	Derivative	Parent	Derivative	
1	Sulfacetamide	3478 3372	3367	1646	--	8,9
2	Sulfadiazine	3390 3310	3279	1653	--	10,11
3	Sulfamerazine	3442 3333	3378	1630	--	12,13
4	Sulfamethazine	3401 3300	3333	1644	--	14,15
5	Sulfapyridine	3384 3279	3322	1639	1638	16,17
6	Sulfisomidine	3425 3322	3311	1634	1639[a]	18,19
7	Sulfaproxyline	3431 3350	3378	1633	--	20,21
8	Sulfaguanidine	3367 3305	3367 3300	1621	1633	22,23
9	Sulfamethoxazole	3413 3318	3305	1618	1618	24,25
10	Sulfaphenazole	3390 3300	3419 3317	1631	1629	26,27

a. Very weak.

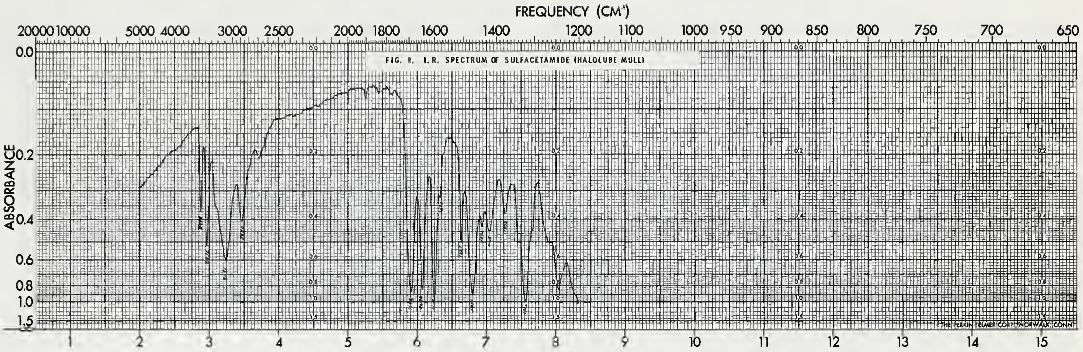


FIG. 8. I.R. SPECTRUM OF SULFACETAMIDE (HALOLUBE MULL)

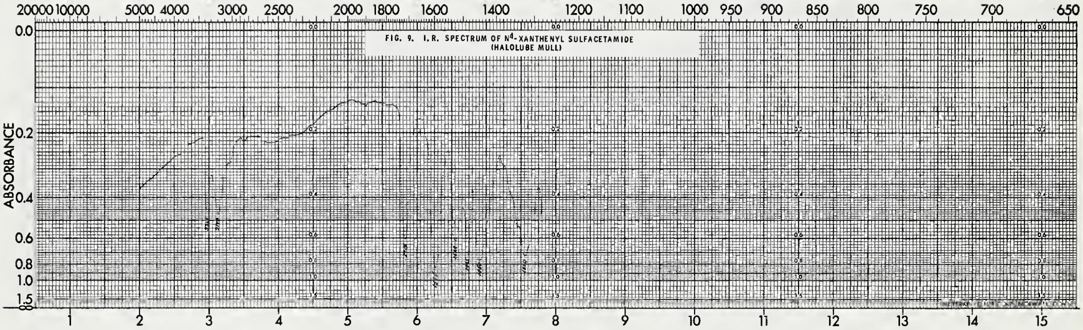


FIG. 9. I.R. SPECTRUM OF N^4-XANTHENYL SULFACETAMIDE (HALOLUBE MULL)

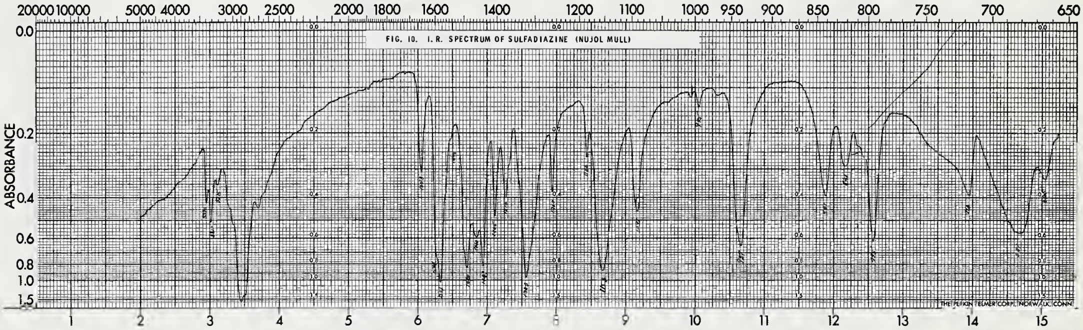


FIG. 10. I.R. SPECTRUM OF SULFADIAZINE (NUJOL MULL)

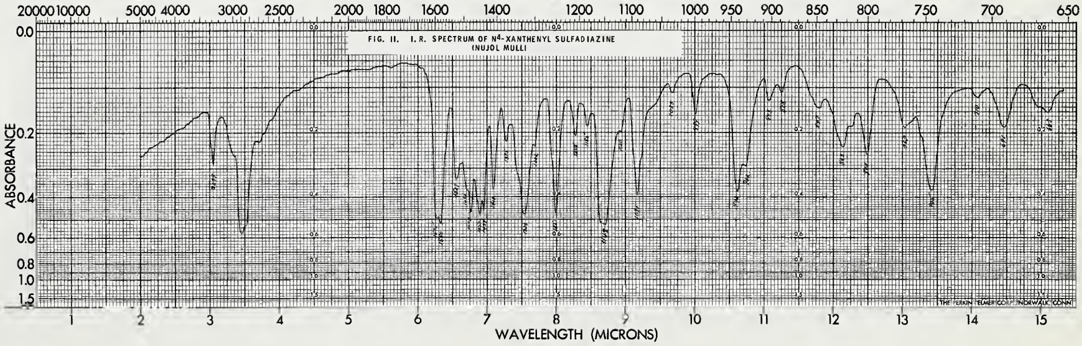


FIG. 11. I.R. SPECTRUM OF N^4-XANTHENYL SULFADIAZINE (NUJOL MULL)

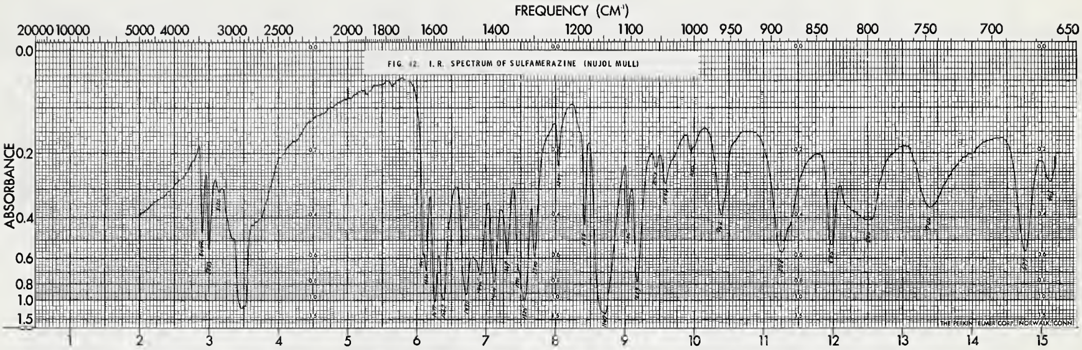


FIG. 12. I. R. SPECTRUM OF SULFAMERAZINE (NUJOL MULL)

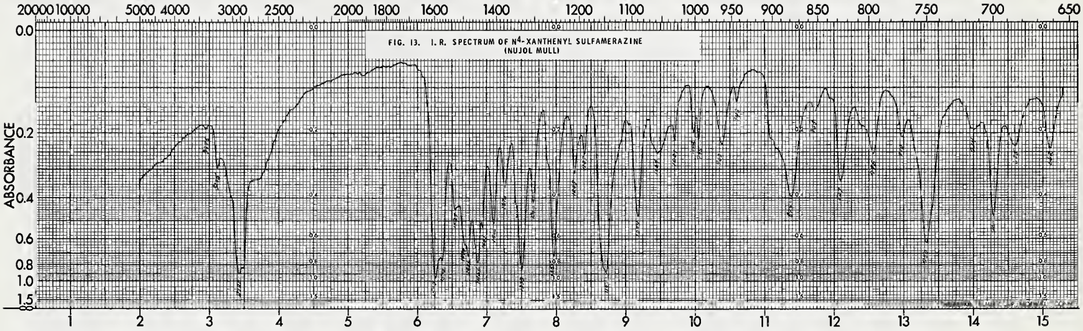


FIG. 13. I. R. SPECTRUM OF N^4-XANTHENYL SULFAMERAZINE (NUJOL MULL)

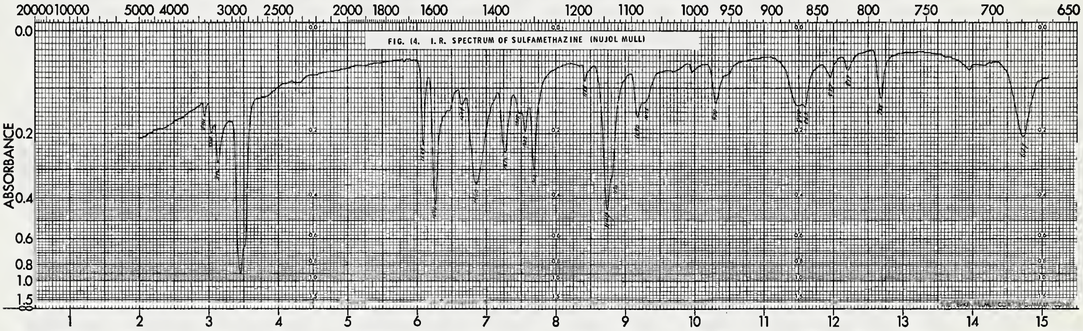


FIG. 14. I. R. SPECTRUM OF SULFAMETHAZINE (NUJOL MULL)

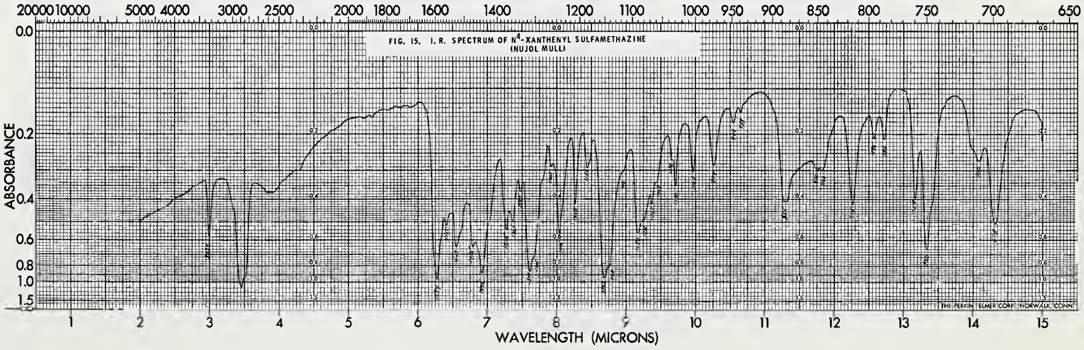


FIG. 15. I. R. SPECTRUM OF N^4-XANTHENYL SULFAMETHAZINE (NUJOL MULL)

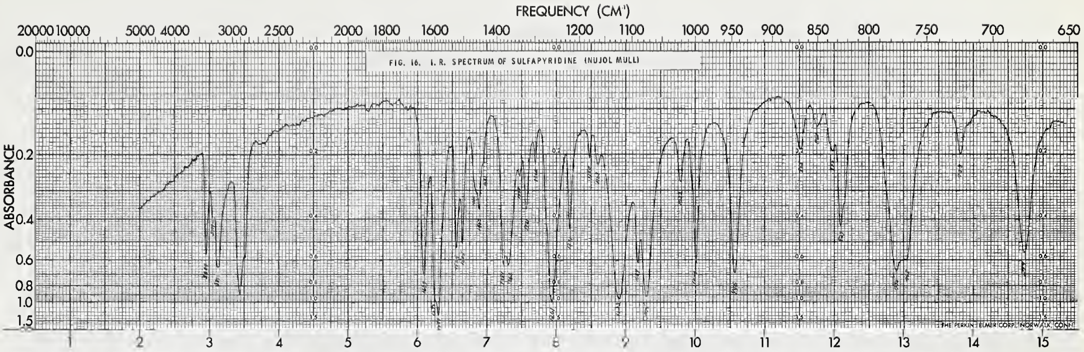


FIG. 16. I. R. SPECTRUM OF SULFAPYRIDINE (NUJOL MULL)

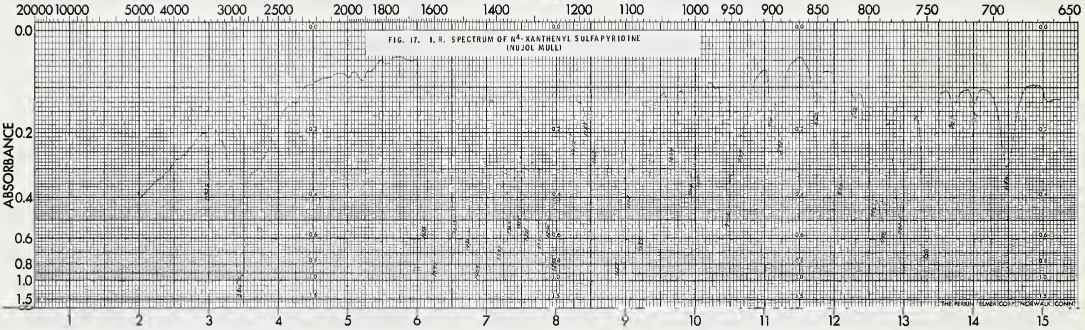


FIG. 17. I. R. SPECTRUM OF N^4-XANTHENYL SULFAPYRIDINE (NUJOL MULL)

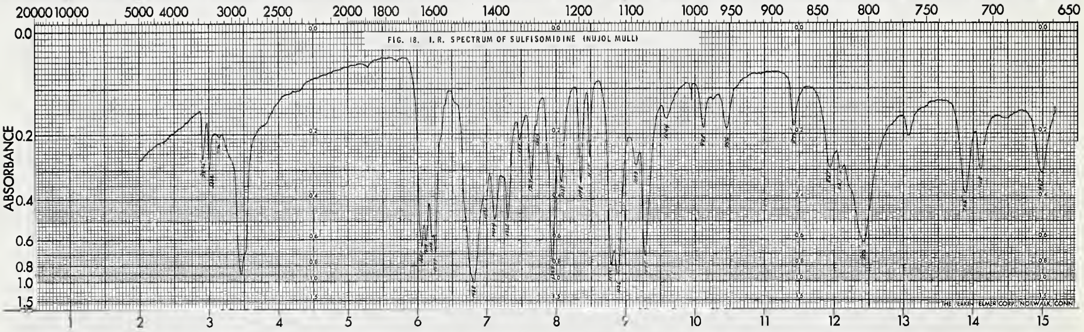


FIG. 18. I. R. SPECTRUM OF SULFISOMIDINE (NUJOL MULL)

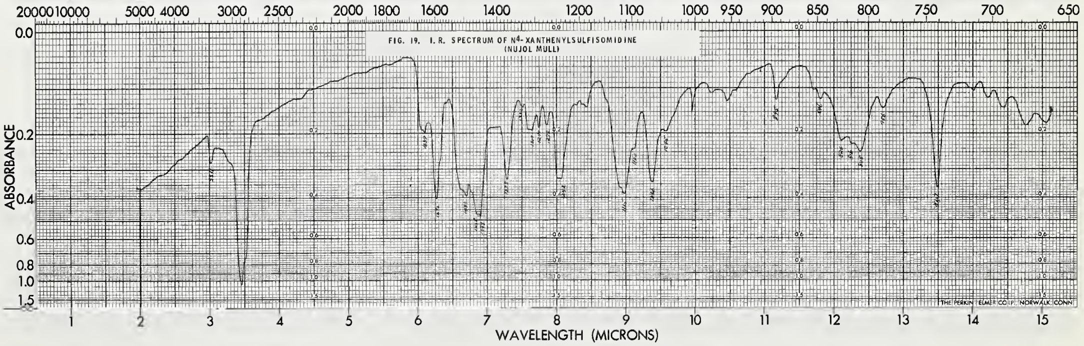


FIG. 19. I. R. SPECTRUM OF N^4-XANTHENYLSULFISOMIDINE (NUJOL MULL)

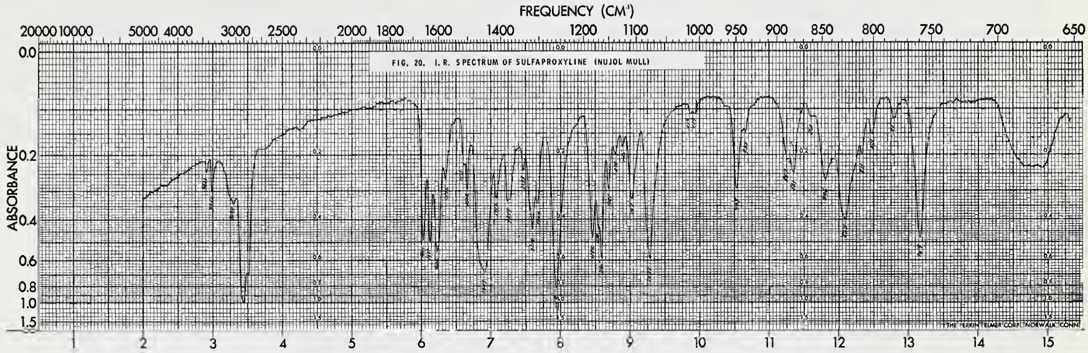


FIG. 20. I.R. SPECTRUM OF SULFAPROXYLINE (NUJOL MULL)

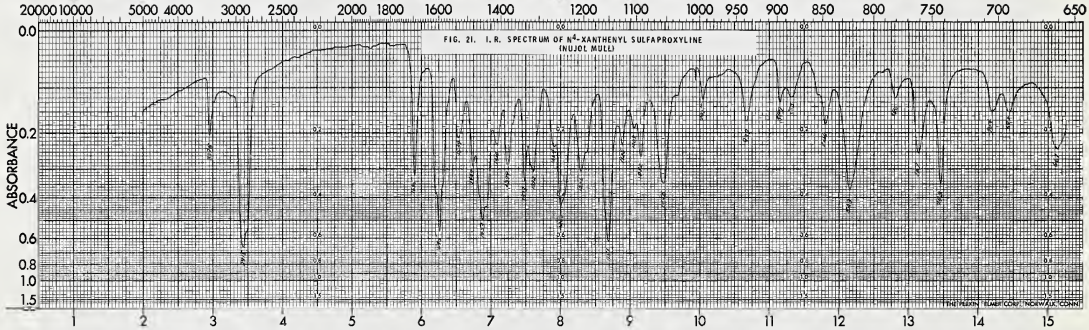


FIG. 21. I.R. SPECTRUM OF N^4-XANTHENYL SULFAPROXYLINE (NUJOL MULL)

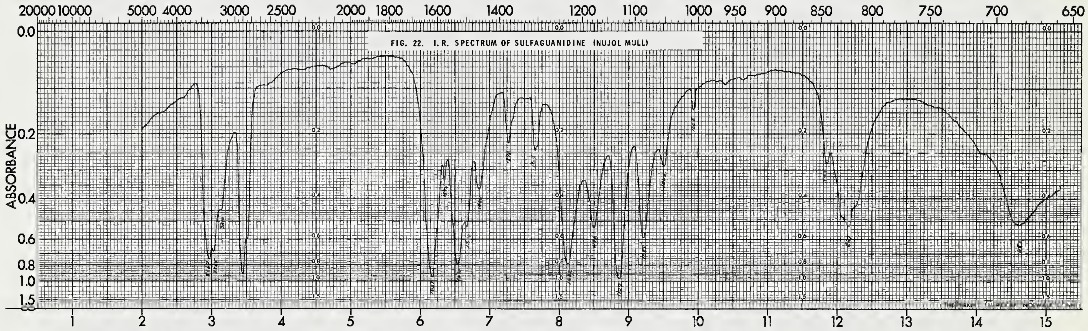


FIG. 22. I.R. SPECTRUM OF SULFAGUANIDINE (NUJOL MULL)

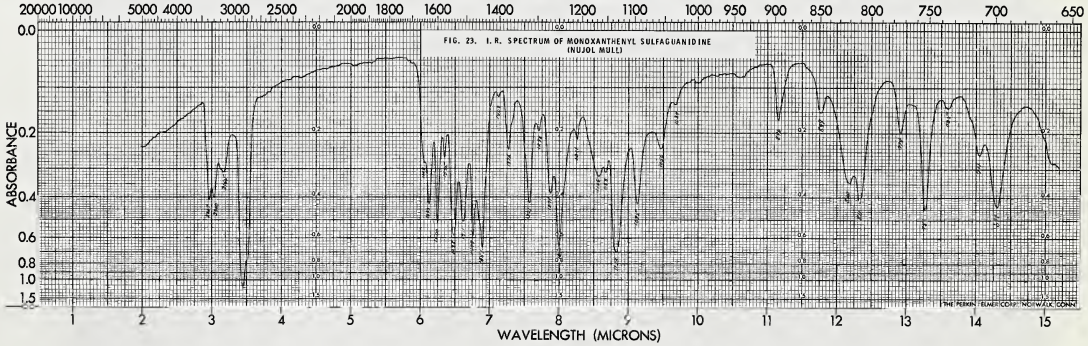


FIG. 23. I.R. SPECTRUM OF MONOXANTHENYL SULFAGUANIDINE (NUJOL MULL)

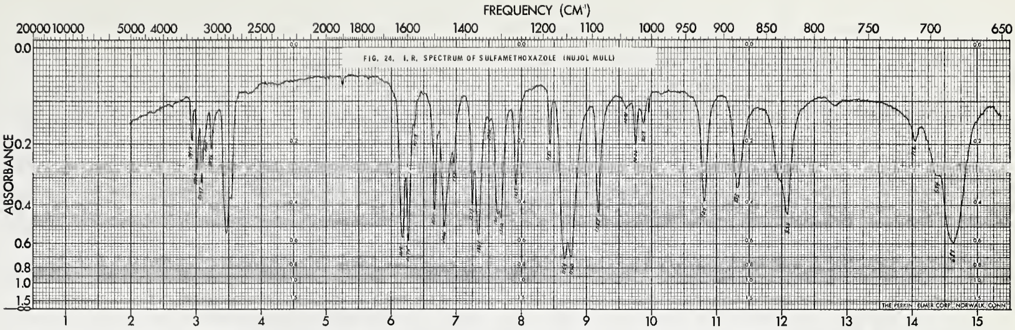


FIG. 24. I.R. SPECTRUM OF SULFAMETHOXAZOLE (NUJOL MULL)

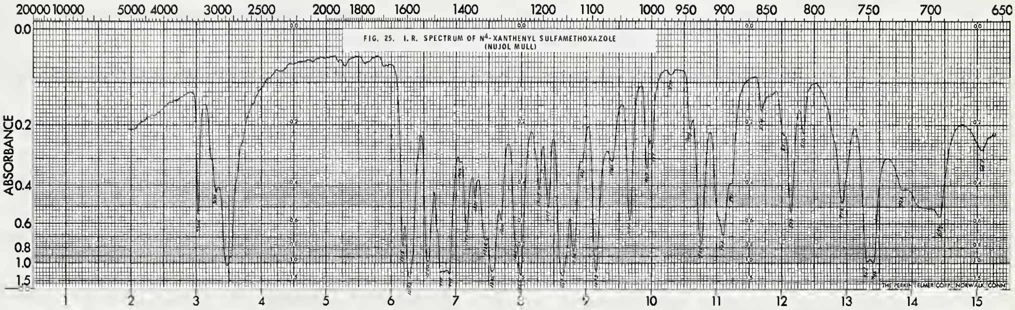


FIG. 25. I.R. SPECTRUM OF N⁴-XANTHENYL SULFAMETHOXAZOLE (NUJOL MULL)

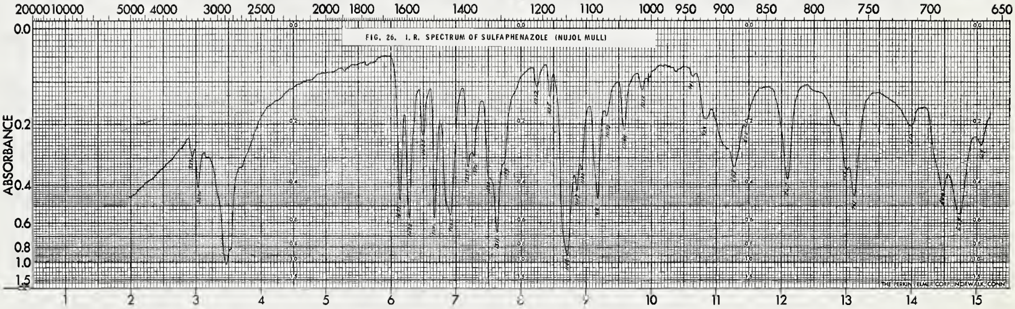


FIG. 26. I.R. SPECTRUM OF SULFAPHENAZOLE (NUJOL MULL)

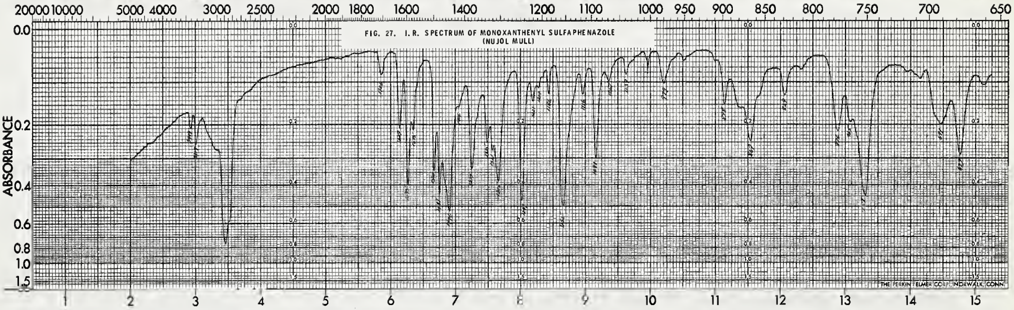


FIG. 27. I.R. SPECTRUM OF MONOXANTHENYL SULFAPHENAZOLE (NUJOL MULL)

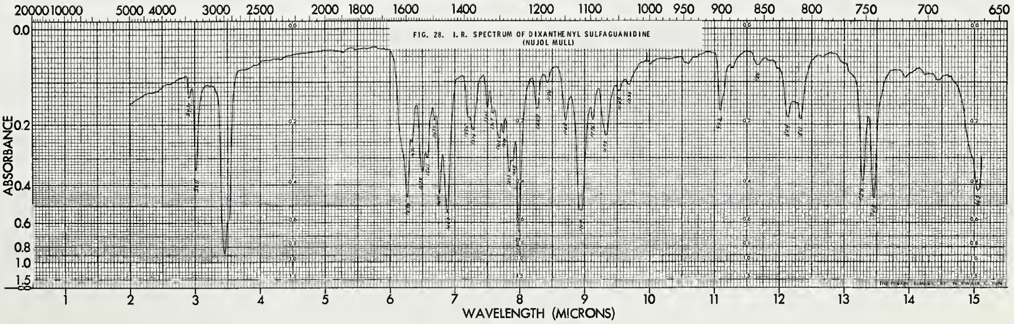
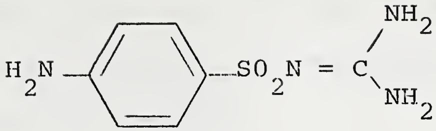


FIG. 28. I.R. SPECTRUM OF DIXANTHENYL SULFAGUANIDINE (NUJOL MULL)

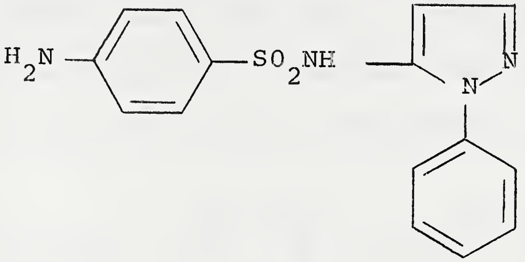


CLXII

some evidence was obtained to show that the reaction of sulfaguanidine with xanthydrol leads to a mixture of a mono- and dixanthenyl derivative. The monosubstituted product was obtained by recrystallization from pyridine-n-hexane at room temperature. A comparable purification from pyridine-water, however, afforded a small amount of a compound which melted approximately 5° higher and which corresponded closely in its analysis with that calculated for the dixanthenyl derivative. The infrared spectrum (Figure 28) still revealed two bands in the 3500-3300 cm^{-1} range, but the band at 1621 cm^{-1} was now missing. Thus, it would appear that substitution with one or both of the amino groups of the guanidino moiety is probable. A more extensive study of this reaction is essential, however, before a structural assignment can be made for this derivative.

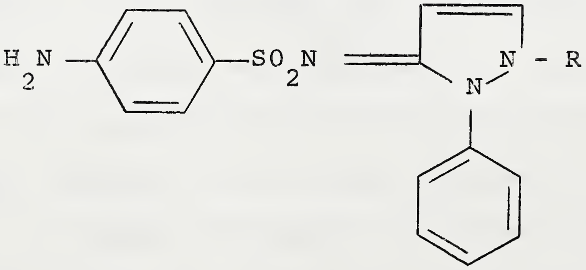
The evidence for the structure of the sulfaphenazole CLXIII derivative is similarly confusing and inconclusive. It will be recalled that this derivative showed a positive nitrous acid test and a weakly positive test with furfural. The results of the sodium pentacyanoaquoferriate test were inconclusive. Upon titration in nonaqueous media a recovery of only approximately 95% was obtained. Also, this was the only derivative that exhibited a slight solubility in 5% sodium hydroxide. The

infrared evidence, as mentioned, suggests the presence of a free amino group. Seydel (140) observed some anomalous results with this compound when he attempted its quantitative determination by the Bratton and Marshall method.



CLXIII

He found that coupling did not proceed normally, and that in fact he recovered the nitroso derivative, nitrosation having occurred at position 4 of the pyrazole ring. The N^1-acetyl derivative of sulfaphenazole, on the other hand, coupled normally. He thus concluded that sulfaphenazole (CLXIII) existed, at least in part, as 5-sulfanilimido-1-phenyl-2H-pyrazoline (CLXIV).

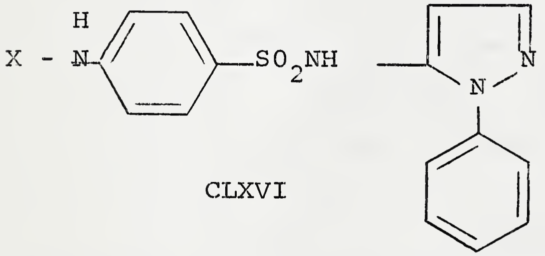


CLXIV, R = H

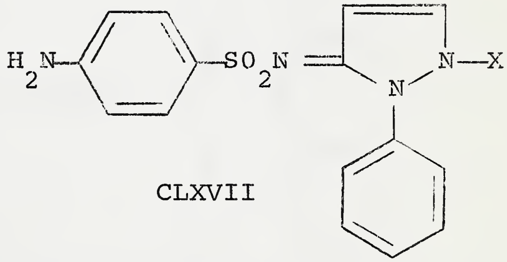
CLXV, R = CH_3

On methylation with methyl iodide or dimethylsulfate in alkaline medium, he recovered only CLXV and could not obtain the N^1-methyl analog even with diazomethane.

On the basis of these findings it appears probable that the derivative obtained in this investigation may in fact be a mixture of CLXVI and CLXVII, with the former predominating. This would explain a low recovery from titration, the positive tests for primary amines and still yield a satisfactory elemental analysis.



CLXVI



CLXVII

X = 9-xanthenyl

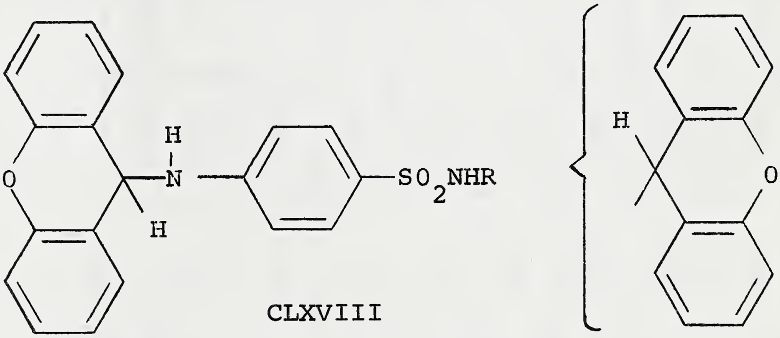
Once again, a more thorough investigation of this reaction is indicated prior to making any structural commitment.

b). Dixanthenyl-N^1-Monosubstituted Sulfanilamides.

According to their elemental analyses, the sulfanilamides listed in Table II each provided the corresponding dixanthenyl derivative when condensed with xanthydrol. These parent compounds differ from those yielding monosubstituted products only with respect to the identity of the group attached at the N^1-position. Consequently, it seemed reasonable to assume that one of the xanthenyl radicals in these derivatives must occupy the N^4-position, in line with the conclusions reached for the monosubstituted products. The above supposition was verified by the similar band characteristics of their infrared spectra. As was true for the monoxanthenyl derivatives, only one band appeared in the NH

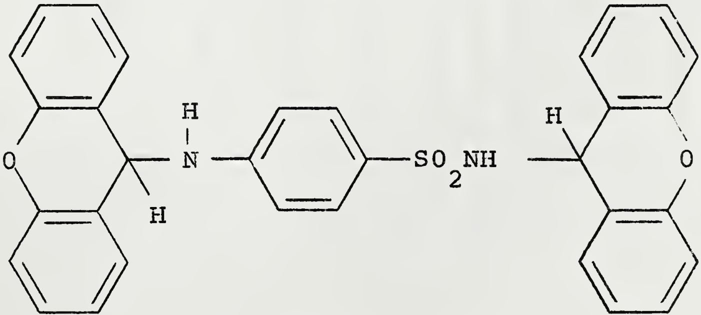
stretching region, and the deformation vibration at 1640 cm^{-1} had disappeared. The band positions corresponding to the aforementioned vibrations are given in Table V, and the original spectra are reproduced in Figures 29 to 45 inclusive.

Based on this evidence, the partial structures of these dixanthenyl derivatives may be represented as follows (structure CLXVIII):



CLXVIII

Assignment of a structure to the dixanthenyl derivative of sulfanilamide itself now posed no problem, since the site of substitution of the second mole of xanthydrol was obvious from published data with primary sulfonamides. This compound is therefore correctly represented as N^1,N^4-dixanthenyl sulfanilamide (CLXIX), and not $3,N^1$-dixanthenyl sulfanilamide (VII) as previously postulated by Phillips and Frank (1).



CLXIX

TABLE V

N-H Stretching and Bending Vibrations of Parent N^1-Monosubstituted Sulfanilamides and their Dixanthenyl Derivatives

Ref. No.	COMPOUND	ν NH (cm^{-1})			δ NH_2 (cm^{-1})		Fig. No.
		Parent		Der.	Parent	Der.	
11	Sulfanilamide	3430	3305	3333	1630	--	29,30
12	Sulfamethizole	3384	3284	3340[a]	1645	--	31,32
13	Sulfaethylthiadiazole	3442	3322	3344	1639	--	34,35
14	Sulfathiazole	3279	3226	3300	1626[b]	--	36,37
15	Sulfamethoxypyridazine	3448	3356	3300	1634	--	38,39
16	Sulfachloropyridazine	3472	3362	3300	1631	--	40,41
17	Sulfisoxazole	3436	3322	3344	1631	--	42,43
18	Sulfadimethoxine	3390	3279	3310	1647	--	44,45

a. 3401 cm^{-1} in chloroform (see Fig. 33)
b. Shoulder.

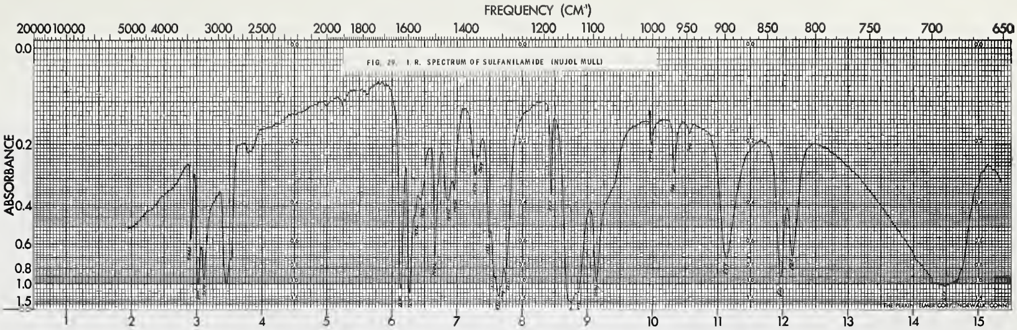


FIG. 29. I.R. SPECTRUM OF SULFANILAMIDE (NUJOL MULL)

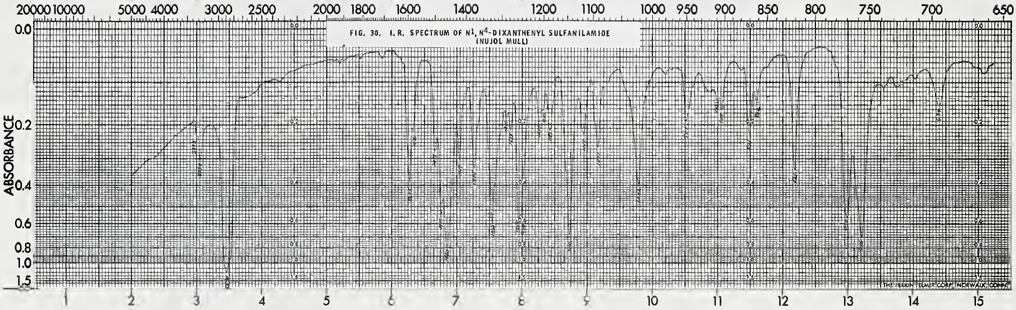


FIG. 30. I.R. SPECTRUM OF N¹, N⁴-DIXANTHENYL SULFANILAMIDE (NUJOL MULL)

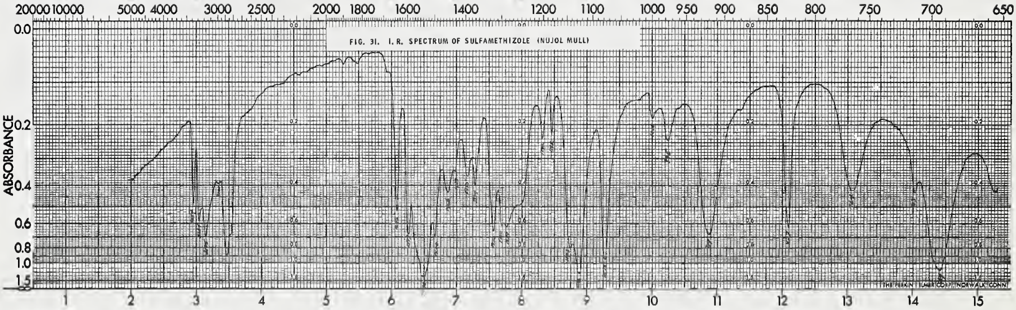


FIG. 31. I.R. SPECTRUM OF SULFAMETHIZOLE (NUJOL MULL)

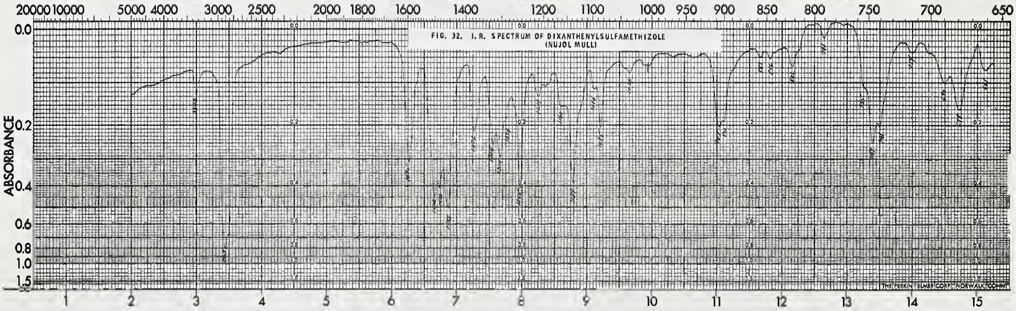


FIG. 32. I.R. SPECTRUM OF DIXANTHENYLSULFAMETHIZOLE (NUJOL MULL)

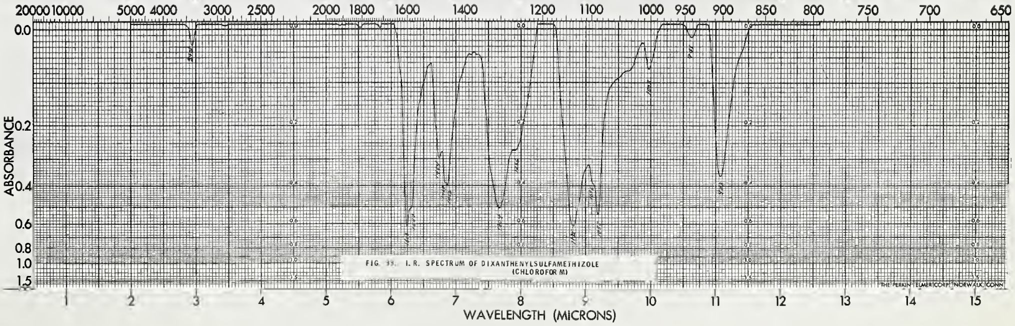


FIG. 33. I.R. SPECTRUM OF DIXANTHENYLSULFAMETHIZOLE (CHLOROFORM)

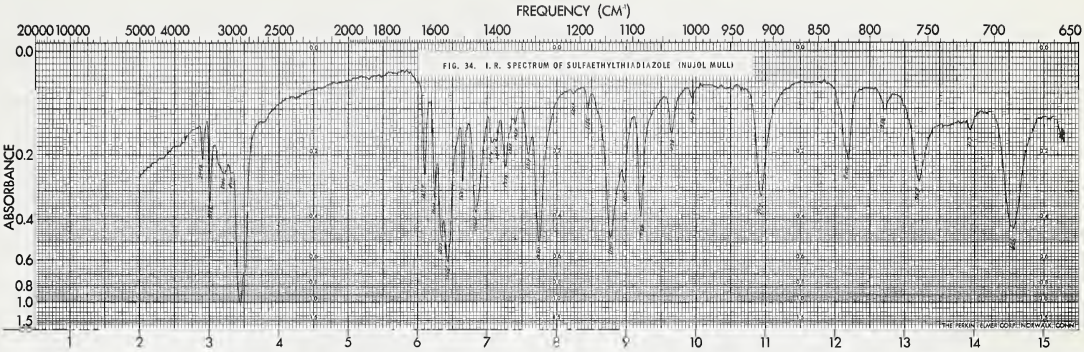


FIG. 34. I.R. SPECTRUM OF SULFAETHYLTHIADIAZOLE (NUJOL MULL)

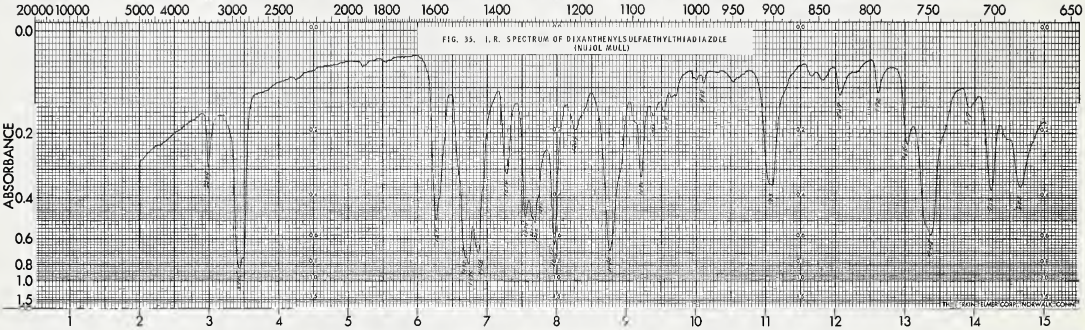


FIG. 35. I.R. SPECTRUM OF DIXANTHENYLSULFAETHYLTHIADIAZOLE (NUJOL MULL)

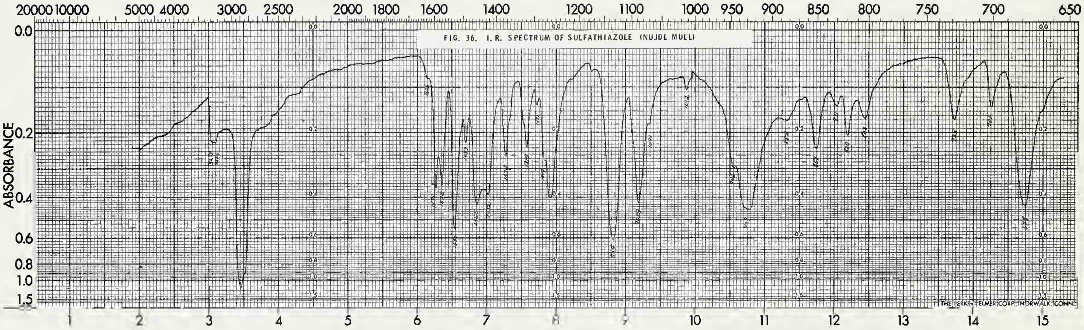


FIG. 36. I.R. SPECTRUM OF SULFATHIAZOLE (NUJOL MULL)

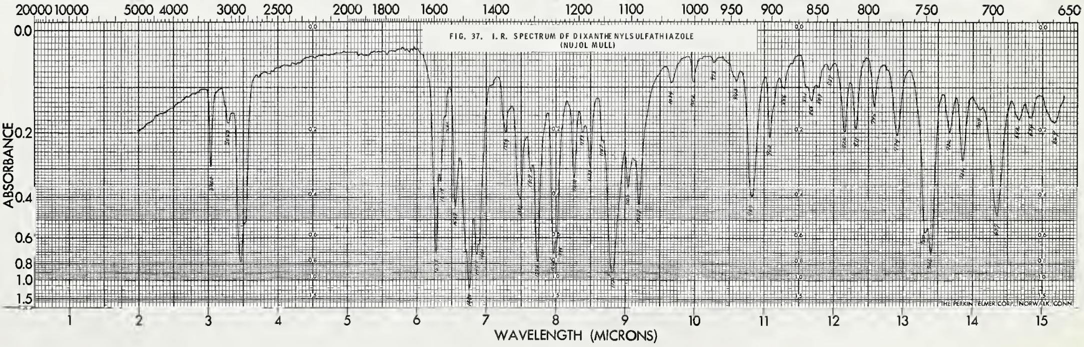


FIG. 37. I.R. SPECTRUM OF DIXANTHENYLSULFATHIAZOLE (NUJOL MULL)

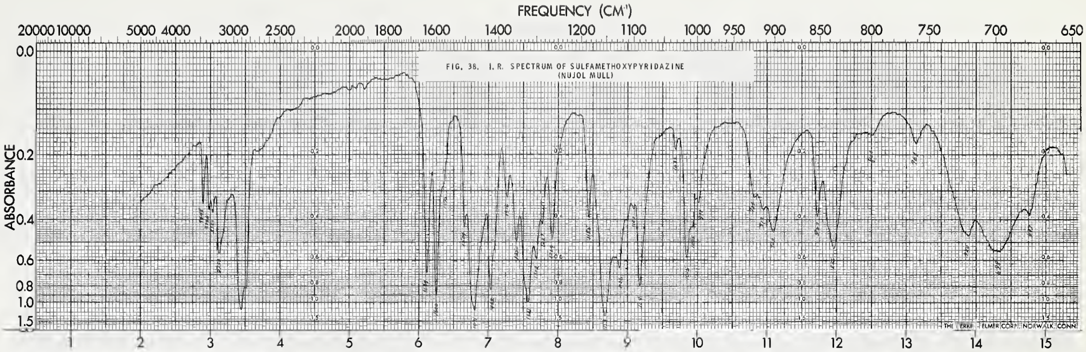


FIG. 38. I.R. SPECTRUM OF SULFAMETHOXYPYRIDAZINE (NUJOL MULL)

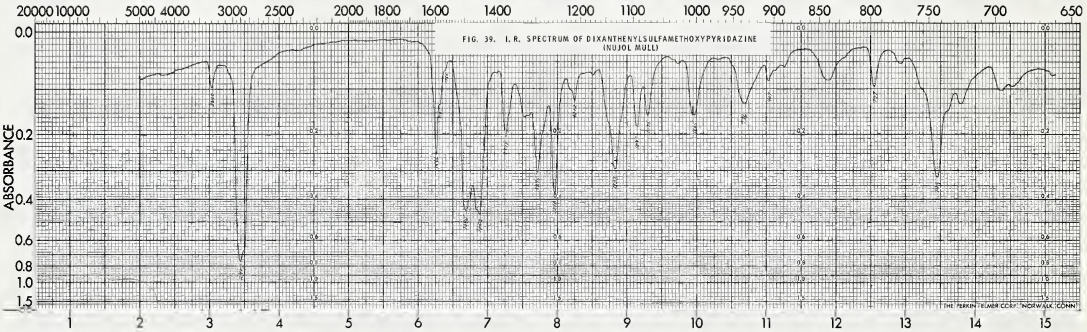


FIG. 39. I.R. SPECTRUM OF DIXANTHENYLSULFAMETHOXYPYRIDAZINE (NUJOL MULL)

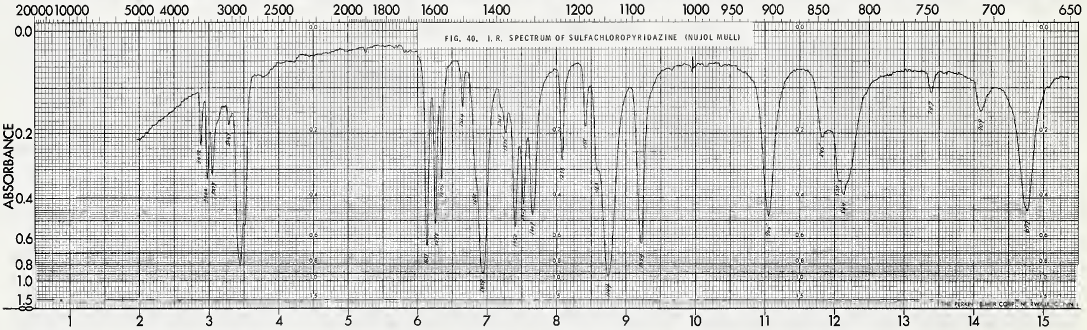


FIG. 40. I.R. SPECTRUM OF SULFACHLOROPYRIDAZINE (NUJOL MULL)

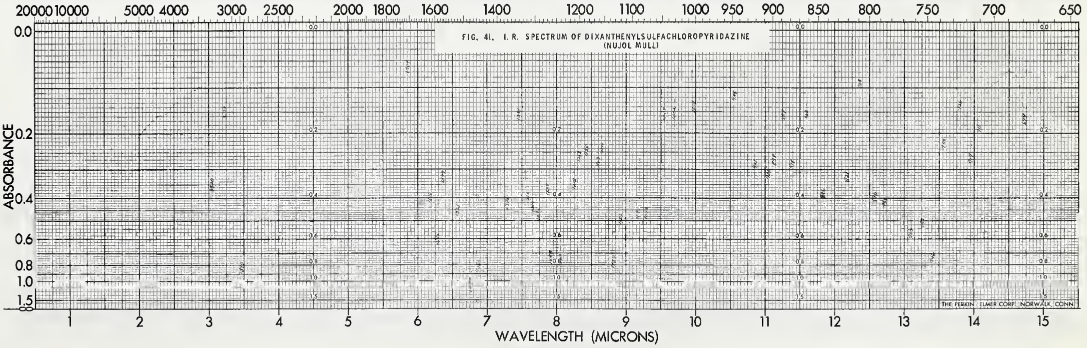


FIG. 41. I.R. SPECTRUM OF DIXANTHENYLSULFACHLOROPYRIDAZINE (NUJOL MULL)

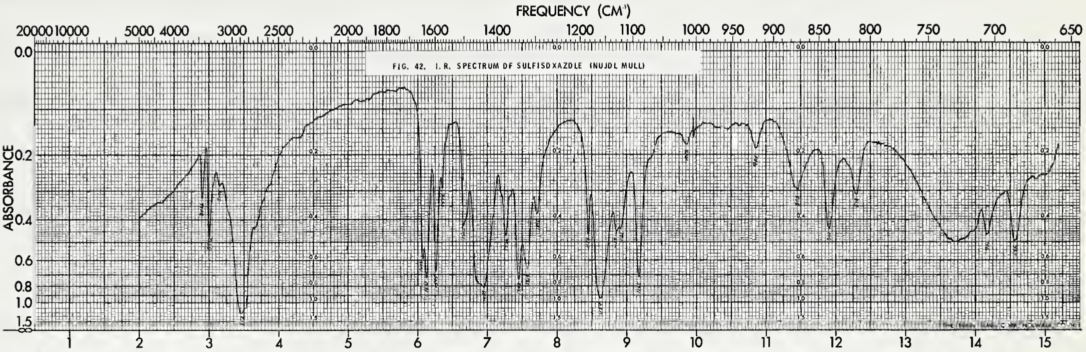


FIG. 42. I.R. SPECTRUM OF SULFISOXAZOLE (NUJOL MULL)

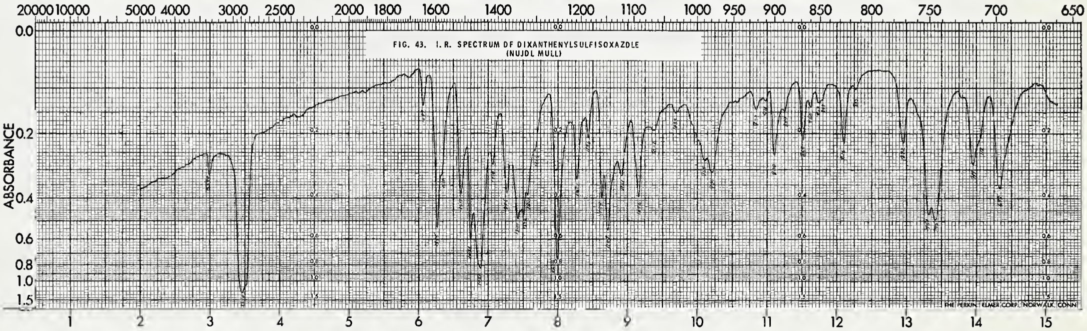


FIG. 43. I.R. SPECTRUM OF DIXANTHENYLSULFISOXAZOLE (NUJOL MULL)

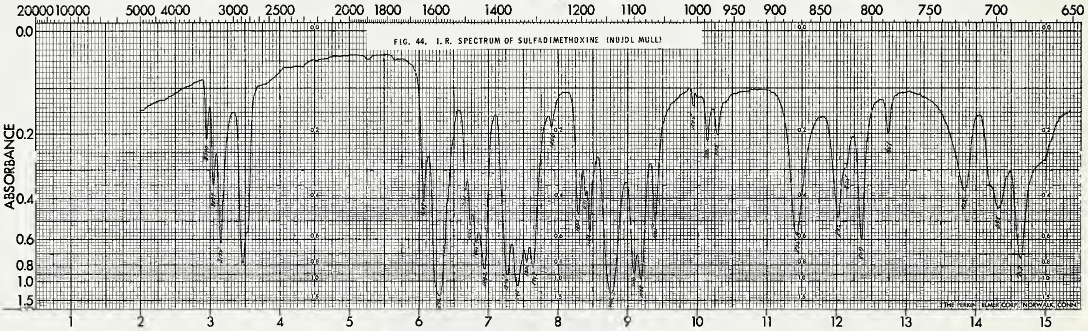


FIG. 44. I.R. SPECTRUM OF SULFADIMETHOXINE (NUJOL MULL)

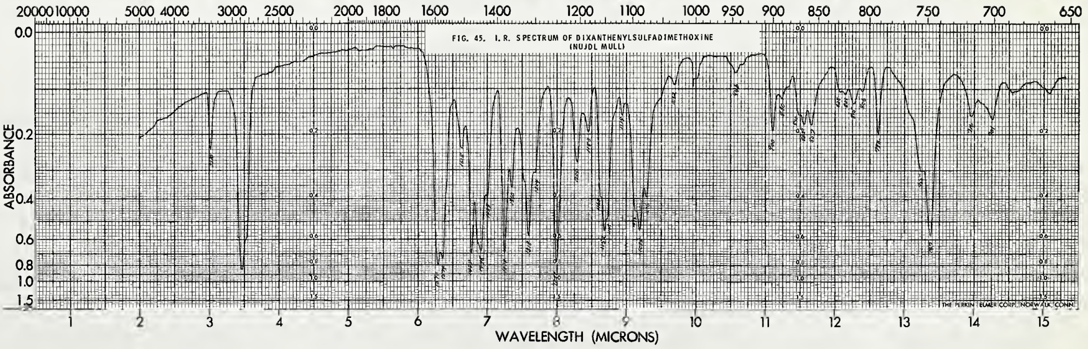
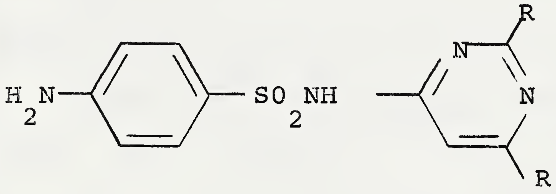


FIG. 45. I.R. SPECTRUM OF DIXANTHENYLSULFADIMETHOXINE (NUJOL MULL)

As opposed to the monoxanthenyl derivatives, each of the remaining dixanthenyl derivatives in Table II, with the exception of that of sulfadimethoxine, failed to titrate as acids in non-aqueous media. This strongly suggested that the acidic hydrogen in these derivatives had been replaced by the second xanthenyl radical.

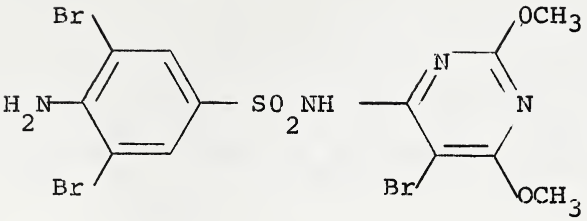
The fact that the dixanthenyl derivative of sulfadimethoxine (CLXX) was still titratable, left the 5-position on the pyrimidine ring as the only logical site for substitution by the second mole of xanthydrol. This position has been shown to be very reactive towards electrophilic substitution when the 2 and 4 positions are occupied by strongly ortho-para directive groups (141). It may be recalled that the methyl analog of this compound, sulfisomidine (CLXXI) yielded only a mono-xanthenyl derivative.



CLXX, R = OCH_3

CLXXI, R = CH_3

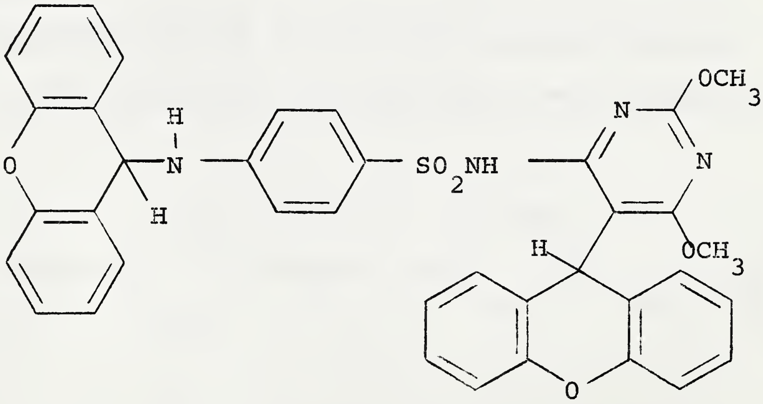
This would suggest that the methyl groups do not activate the 5-position sufficiently to result in electrophilic substitution at this site. Evidence to this effect came from the fact that the former compound CLXX, on bromination, afforded a tribromo derivative (CLXXII). Sulfisomidine (CLXXI), on the other hand, has been reported to take up only two moles of bromine (142).



CLXXII

Treatment of CLXXII with xanthydrol yielded only the original starting material.

Therefore, the structure of the dixanthenyl derivative of sulfadimethoxine must be that shown in formula CLXXIII.

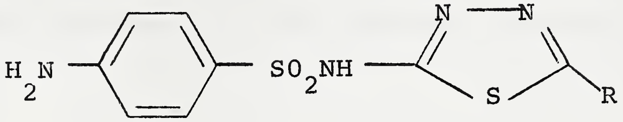


CLXXIII

Data in support of N^1 substitution for the remainder of the members in this series was obtained from experiments with various analogs of these compounds.

Sulfamethizole (CLXXIV) and sulfaethylthiadiazole (CLXXV), the

first two compounds in this category, both contain a 2-alkyl thiadiazole substituent in the N^1-position.

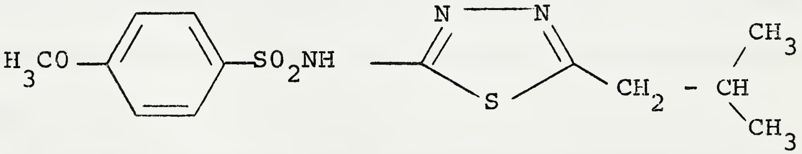


CLXXIV, $R = CH_3$

CLXXV, $R = C_2H_5$

As mentioned earlier, none of the derivatives in this group were titratable, although the parent compounds afford quantitative recoveries.

Condensation of the structurally related material, isobuzole (CLXXVI), with xanthydrol, yielded only a mono substituted product.

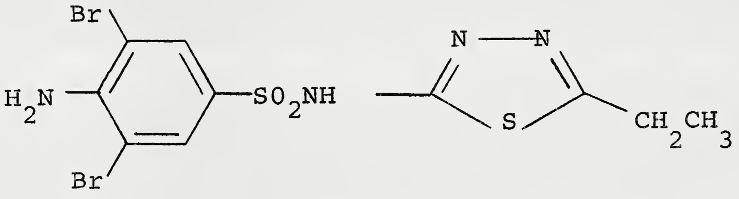


CLXXVI

This derivative, in contrast with those previously encountered, was easily purified by recrystallization from ethanol with no apparent signs of decomposition. It also revealed the absence of a titratable acidic hydrogen when compared with the original compound. The failure of the methoxy group to induce ring

substitution, furnished added support in favor of substitution at the N^4- position.

Similarly, bromination of sulfaethylthiadiazole (CLXXV), to give CLXXVII, followed by reaction with xanthydrol afforded a monoxanthenyl derivative. It, likewise, was easily purified from ethanol in the normal manner, and also failed to titrate. The steric effects of the bromine atoms on the amino group, observed earlier, were verified by the results of the latter reaction. Lack of reactivity at this site (N^4) was evident from a comparison of the infrared spectrum of the dibromo compound CLXXVII (Figure 46) and that of its xanthenyl derivative (Figure 47). The spectral features characteristic of the primary amino function appeared in both spectra.



CLXXVII

Also of great interest, was the apparent stability of the derivatives of CLXXVI and CLXXVII, neither of which involve reaction with a primary amino group. This observation served to isolate the C^9(xanthenyl)-N^4(sulfanilamide) bond as the structural feature responsible for the instability of these compounds. Furthermore, it pointed to the stronger nature of the bond involving the second mole of xanthydrol.

Studies on several commercially available N^4- derivatives of

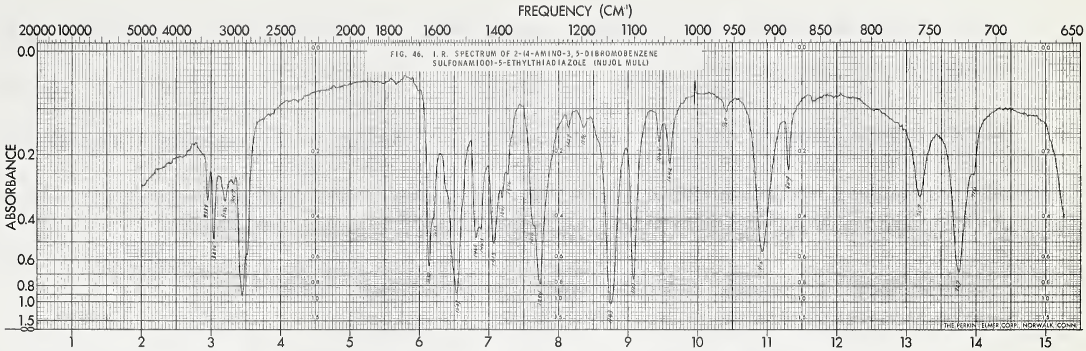


FIG. 46. I. R. SPECTRUM OF 2-(4-AMINO-3, 5-DIBROMOBENZENE SULFONAMIDO)-5-ETHYLTHIADIAZOLE (NUJOL MULL)

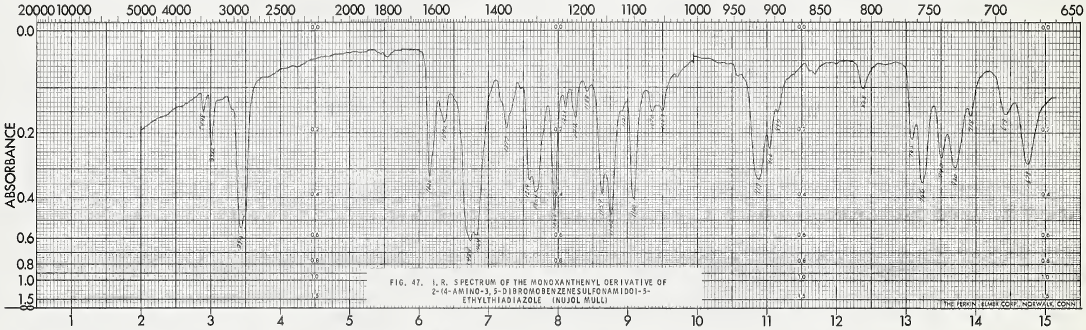


FIG. 47. I. R. SPECTRUM OF THE MONOXANTHENYL DERIVATIVE OF 2-(4-AMINO-3, 5-DIBROMOBENZENESULFONAMIDO)-5-ETHYLTHIADIAZOLE (NUJOL MULL)

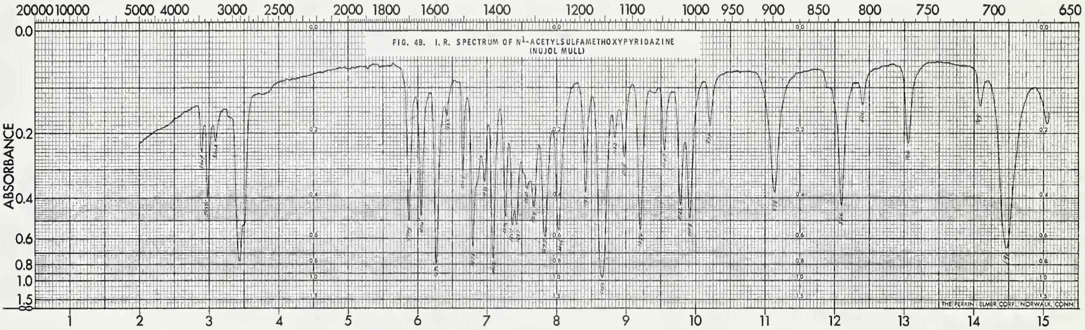


FIG. 48. I. R. SPECTRUM OF N^1-ACETYLSULFAMETHOXYPYRIDAZINE (NUJOL MULL)

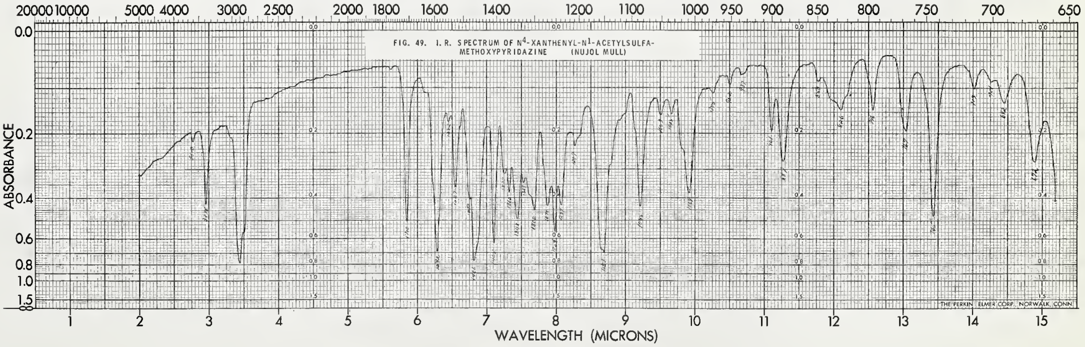
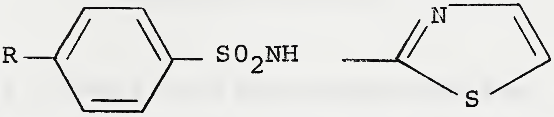


FIG. 49. I. R. SPECTRUM OF N^4-XANTHENYL-N^1-ACETYLSULFAMETHOXYPYRIDAZINE (NUJOL MULL)

sulfathiazole (CLXXVIII), the next member in this series, provided supportive evidence in favor of N^1-substitution. Phthalyl (CLXXIX), succinyl (CLXXX) and p-nitro (CLXXXI) sulfathiazole when condensed with xanthydrol, each provided only monosubstituted derivatives.



CLXXVIII, R = $-NH_2$

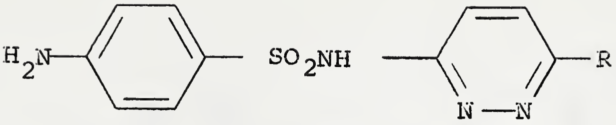
CLXXIX, R = $-NHCO-C_6H_4-\underline{o}-(COOH)$

CLXXX, R = $-NHCOCH_2CH_2COOH$

CLXXXI, R = $-NO_2$

The derivatives of CLXXIX and CLXXX titrated in a mole-to-mole ratio, as opposed to the parent compounds, each of which behave as a diprotic acid. The derivative of the p-nitro compound (CLXXXI), on the otherhand, failed to titrate, as expected.

Reinforcing evidence of a different nature was acquired from studies with an analog of sulfamethoxypyridazine (CLXXXII) and sulfachloropyridazine (CLXXXIII), the succeeding two members falling in this category.

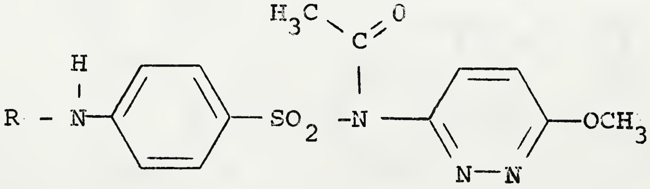


CLXXXII, R = OCH_3

CLXXXIII, R = Cl

Condensation of N^1-acetyl sulfamethoxypyridazine* (CLXXXIV) with xanthydrol yielded only a monoxanthenyl derivative.

In line with similar previous observations, this derivative was very unstable and difficult to purify. The infrared spectrum of the parent compound showed NH stretching vibrations at 3454 and 3356 cm^{-1} and a strong NH deformation band at 1655 cm^{-1} (Figure 48). The spectrum of the derivative, on the other hand, exhibited only one band in the former region at 3378 cm^{-1}, and the deformation band was absent (Figure 49). Based on this evidence, the N^4-xanthenyl structure (CLXXXV) may be assigned to this derivative.



CLXXXIV, R = H

CLXXXV, R = 9-xanthenyl

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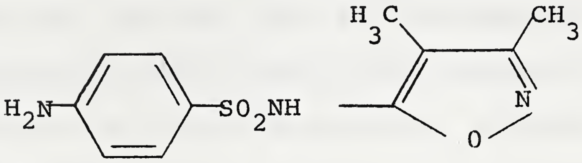
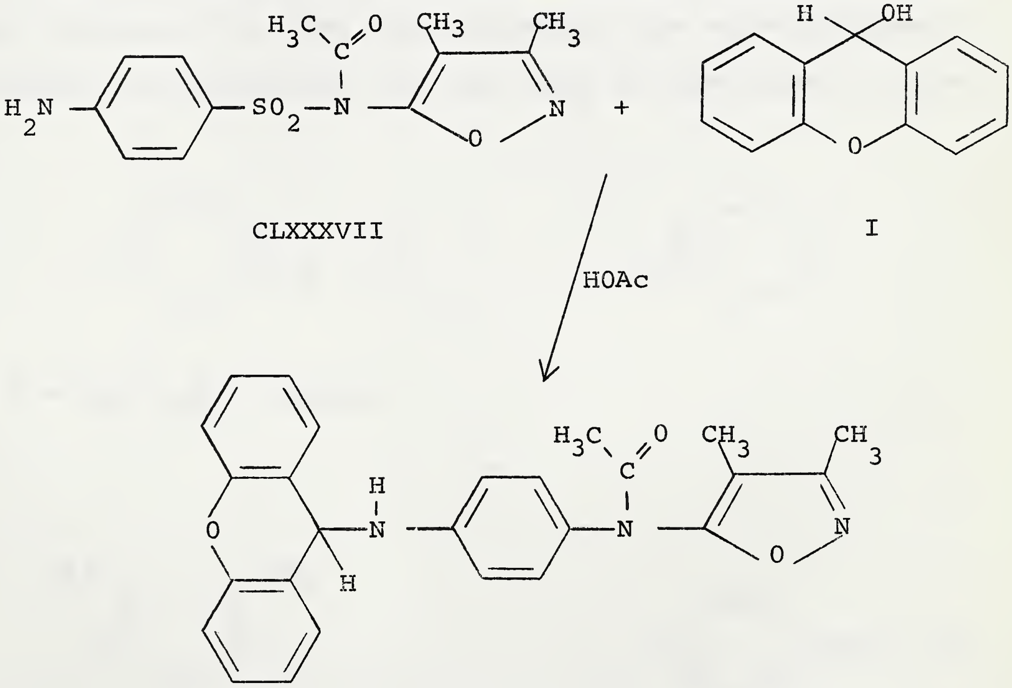
* Available commercially.

Similar results were obtained with an analog of sulfisoxazole (CLXXXVI), the last compound in this series.



CLXXXVI

Treatment of N^1-acetylsulfisoxazole* (CLXXXVII) with xanthydrol (I), afforded the corresponding N^4-xanthenyl derivative (CLXXXVIII).



CLXXXVII

I

CLXXXVIII

---

* Available commercially.

[illegible]

[illegible]

[illegible]

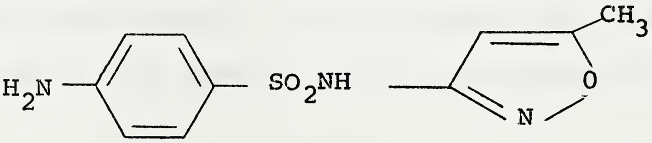
[illegible]

[illegible]

[illegible]

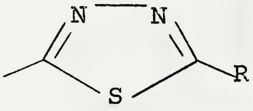
This compound (CLXXXVIII), was also difficult to purify and the infrared spectrum again pointed to amino substitution (see Figures 50 and 51).

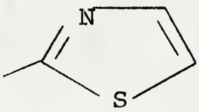
Of interest was the fact that sulfamethoxazole (CLXXXIX), which also contains an alkyl substituted isoxazole ring at N^1, although attached at a different position, afforded only a monoxanthenyl derivative.



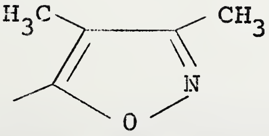
CLXXXIX

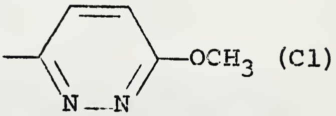
Thus, it may be said that the following four heterocyclic ring systems are in some way influencing the amide hydrogen in a manner not encountered with any other N^1-substituent studied.





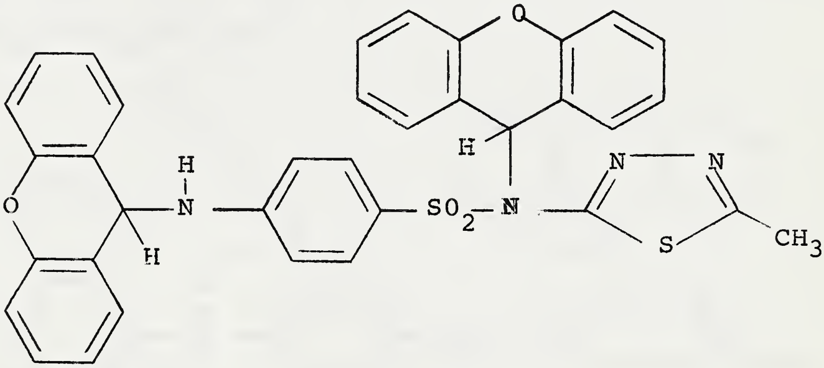
R = CH_3, C_2H_5, isobutyl





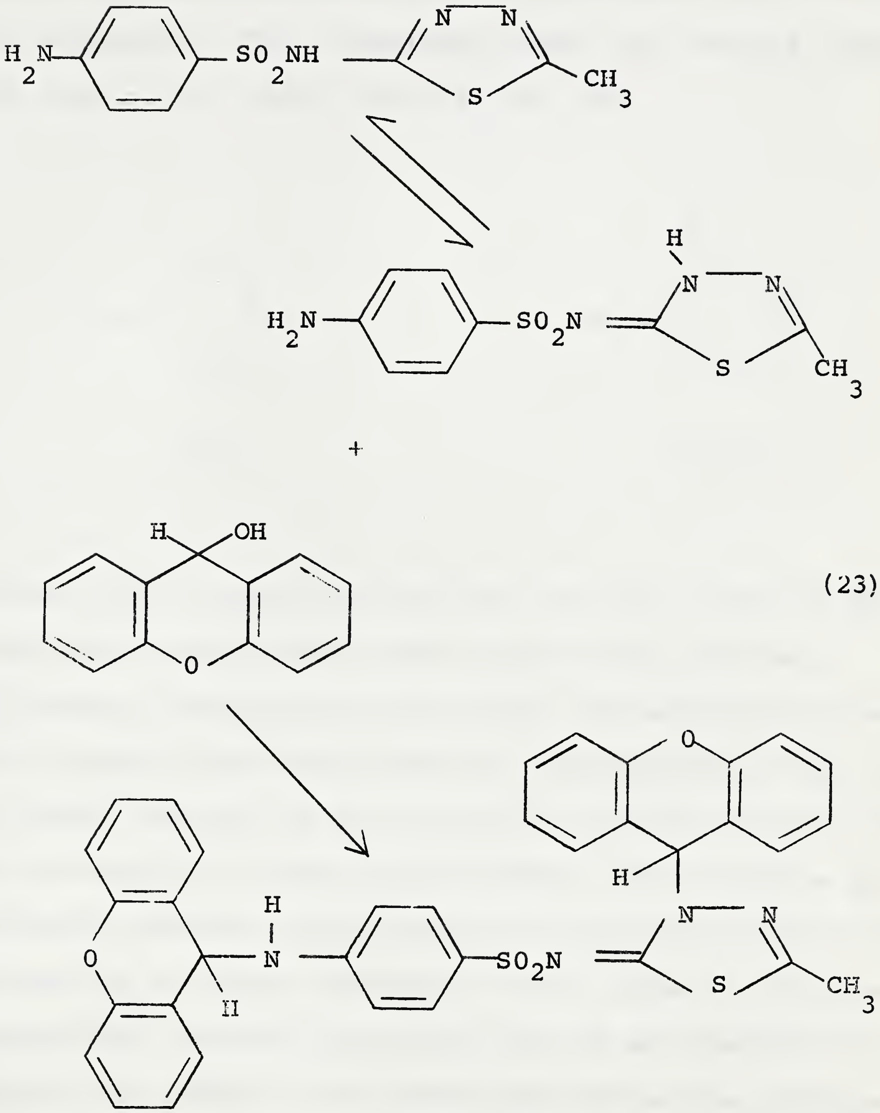
The preceding evidence is conclusive to the point that the amide hydrogen is no longer available for titration. The involvement of this hydrogen is further substantiated by the loss of reactivity at this site upon acetylation.

The disappearance of the amide hydrogen in the derivatives may be accounted for in two ways. First of all, substitution of this hydrogen may have occurred at N^1, thus furnishing N^1,N^4-dixanthenyl derivatives. On this basis, the derivative of sulfamethizole, for example, would be represented as CXC.



CXC

Secondly, the disappearance of the acidic hydrogen may be accounted for by assuming that reaction had occurred from a tautomeric imino form. Taking the same example, CXCI would be the resultant product (equation 23).



(23)

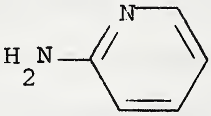
CXCI

A similar argument can be developed for each of the other compounds in this class. Either structure CXC or CXCI would explain the failure of these derivatives to titrate as acids.

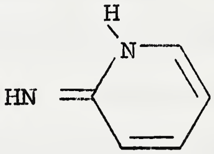
Studies on prototropic tautomerism of heteroaromatic

[illegible]

compounds have revealed that α- and γ- amino derivatives of N-hetero aromatic compounds exist in the amino form, and not as imino compounds. Thus, 2-aminopyridine, for example, exists as CXCII and not as CXCIII (143,144,145,146).



CXCII



CXCIII

Sheinker (146), explained this fact by application of the law of acid-base equilibrium to the amine-imine tautomeric equilibrium. According to his theory, the existence of heterocyclic amines in the amino form is a consequence of the considerably higher basicity of the extra-ring nitrogen atom in comparison with the basicity of the ring-nitrogen. This causes a strong, practically complete, displacement of the equilibrium in the direction of the amino tautomeric form.* Based on this concept, Sheinker (147) further postulated that it was possible for a change in the ratios of the basicities of the two nitrogen atoms responsible for the tautomerism, the ring and the extra-ring nitrogens, to have an effect on the amine-imine tautomeric equilibrium. Accordingly, he predicted that such a change could be realized by replacement of one of the hydrogen atoms of the amino group by electronegative groups such as acid radicals,

* The fact that the amino form retains its aromatic character would explain its favorable position.

which could significantly lower the basicity of, primarily, the extra-ring nitrogen directly bonded to it. In work devoted to this aim, he found that depending on the electron-withdrawing power of the acid radicals (the value of this power was obtained from a measurement of the strengths of the corresponding acids) the resulting heterocyclic amides existed, indeed, not only in the amino form, but also in the imino form or in a mixture of the two tautomeric isomers. From a comparative study of the infrared and ultraviolet spectra of these amides and their methyl derivatives, which have a fixed amino or imino structure, he concluded that the shifting of the tautomeric equilibrium in the direction of the imino form required acyl groups of high "acidizing" power. Furthermore, the minimum value of this power differed for the different heteroaromatic amines.

Thus, the sulfanilic acid radical in N^1-monosubstituted sulfanilamides would be expected to have a variable effect on the amine-imine equilibrium depending on the nature of the heteroaromatic amine. This has indeed been found to be so. Studies have revealed that some sulfonamido derivatives of heteroaromatic systems tend to favor either the amino or the imino forms by a large factor. The above, of course, refers to tautomeric equilibria in solution, since, in the solid state these must exist in either one form or the other. In the former case, solvents in themselves possess the ability to shift the amine-imine equilibrium in favor of one of the tautomeric forms. Sheinker (147) found that the following solvents progressively increased the shift in equilibrium in the direction of the imino tautomer: heptane, dioxane, alcohol, water. It is readily apparent, therefore,

that because of the variable nature of the heteroaromatic group in the N^1-monosubstituted sulfanilamides, the assignment of a preferred tautomeric form requires an extensive study with each individual sulfanilamide. Such studies have been reported for only a very limited number of sulfanilamides.

Ultraviolet and infrared spectroscopic data have been interpreted as favoring the imino form for sulfapyridine (147, 148,149), sulfathiazole (147, 148,149,150), sulfamethizole (150) and sulfadiazine (151). Uno _et_ _al_. (149), from a study of spectral changes on N-deuteration, reached the opposite conclusion for sulfadiazine. To our knowledge, similar studies on other heteroaromatic sulfanilamides have not been reported. As a result of the above studies, several spectral features have been proposed as being useful for distinguishing the two tautomeric forms.

In the infrared region, Sheinker _et_ _al_. (150) reported that these two forms could be distinguished empirically by several spectral differences in the 1000-900 cm^{-1} region. They assigned a 940 cm^{-1} band as being characteristic of the imino $(-\overset{|}{N}-\overset{|}{C} = N-SO_2-)$ grouping, and bands at 1040 cm^{-1} and 860-850 cm^{-1} as being typical of the amino $(-N=\overset{|}{C}-\overset{|}{N}-SO_2-)$ structure. Uno _et_ _al_. (149) have shown, however, that the presence of the 940 cm^{-1} band in pyrimidine derivatives, such as sulfadiazine, cannot be interpreted as signifying the imino form.

An examination of the infrared spectra of the xanthenyl derivatives prepared in this study revealed the presence of one or more bands in the 940 cm^{-1} region in each of the derivatives. However, the spectrum of xanthydrol itself (Figure 52) showed several bands in this region, one of which is probably related to

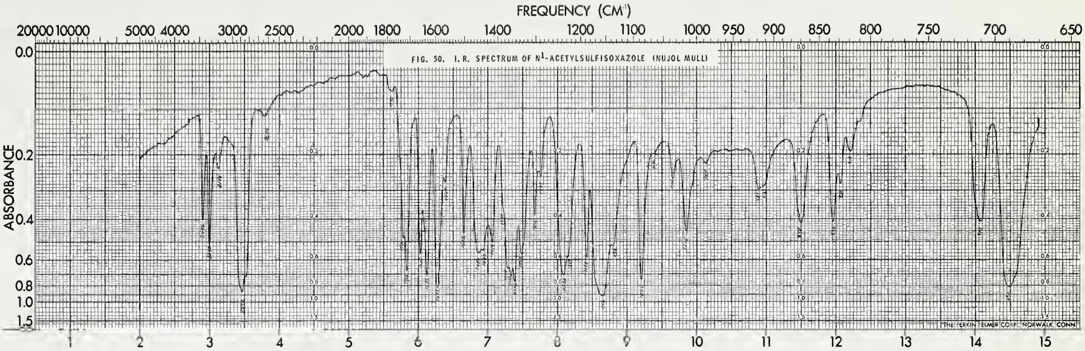


FIG. 50. I.R. SPECTRUM OF N^1-ACETYLSULFISOXAZOLE (NUJOL MULL)

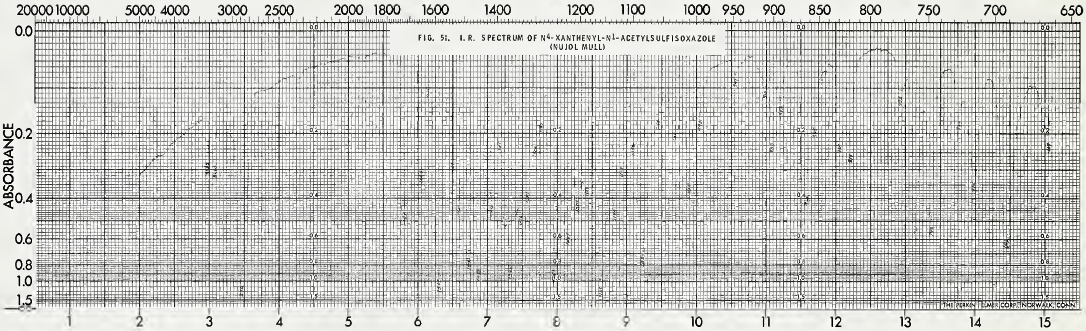


FIG. 51. I.R. SPECTRUM OF N^4-XANTHENYL-N^1-ACETYLSULFISOXAZOLE (NUJOL MULL)

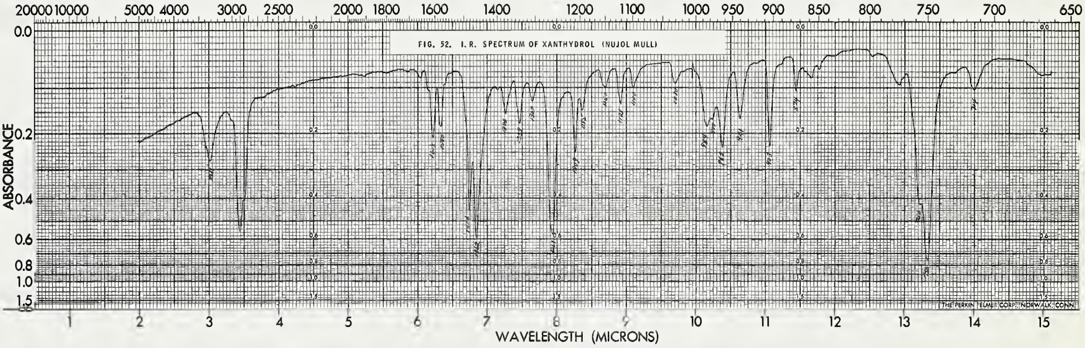


FIG. 52. I.R. SPECTRUM OF XANTHYDROL (NUJOL MULL)

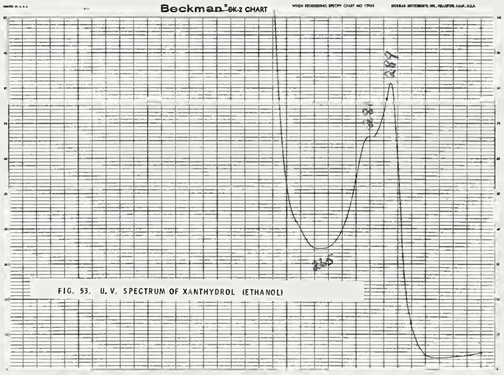


FIG. 53. U.V. SPECTRUM OF XANTHYDROL (ETHANOL)

the C-O stretching vibration (152). Consequently, this band would be expected to appear in the spectra of all the xanthenyl derivatives and thus render interpretation in this region impossible.

In the ultraviolet region, it has been revealed that there is one maximum for the amino $(-N{=}\overset{|}{C}{-}\overset{|}{N}{-}SO_2)$ form, and two maxima in the spectrum of the imino $(-\overset{|}{N}{-}\overset{|}{C}{=}N{-}SO_2{-})$ form (148,150,153). However, once again it became obvious that this empirical criterion could not be extended beyond the N-methyl derivatives from which this test was derived. Xanthydrol itself possesses two maxima in the ultraviolet region (Figure 53) and thus the spectra of the xanthenyl derivatives would be expected to be complicated by these absorptions. A comparison of several ultraviolet spectra verified these suspicions.

Sulfathiazole, which has been shown to exist in the imino form, showed two maxima (Figure 54) one of which appeared in the same region as the maximum in xanthydrol. Its dixanthenyl derivative, on the other hand, revealed four maxima (Figure 55). Sulfamethoxy-pyridazine exhibited one maximum in the ultraviolet (Figure 56), whereas its dixanthenyl derivative showed two maxima (Figure 57). The latter could be interpreted as signifying an amino and imino structure respectively, or the second maximum in the derivative could be attributed to the xanthenyl radicals, and accordingly the amino form assigned to the parent compound and the derivative. Thus, the application of this empirical criterion was also abandoned.

The relative position of the SO_2 symmetric stretching frequency, which normally occurs in the 1170-1130 cm^{-1} region, has

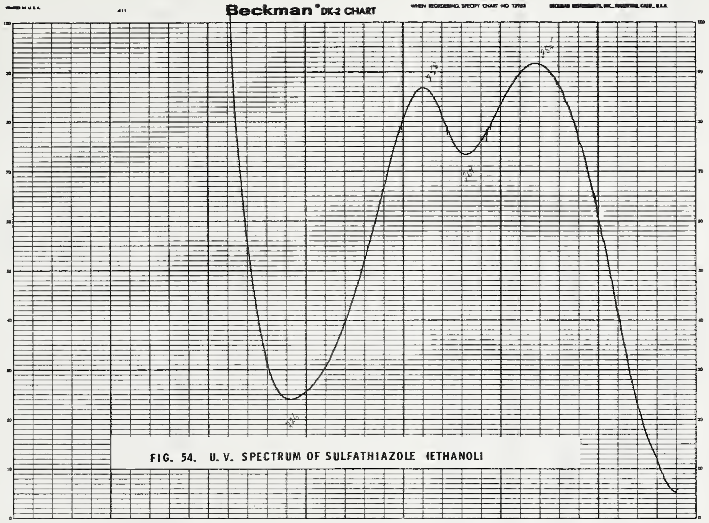


FIG. 54. U. V. SPECTRUM OF SULFATHIAZOLE (ETHANOL)

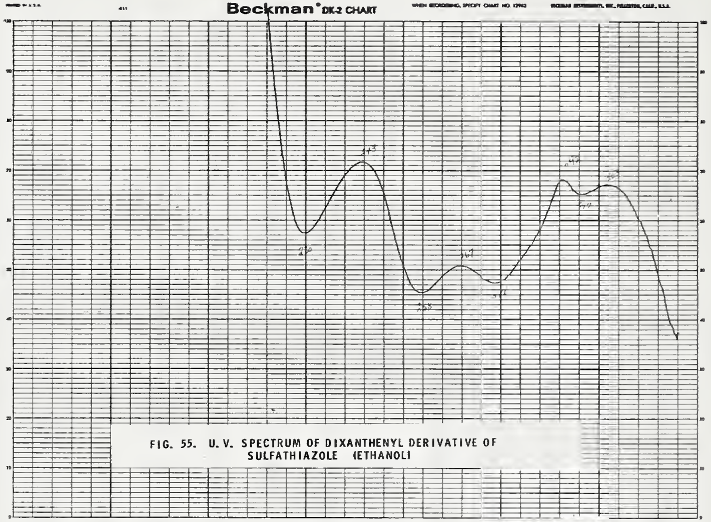


FIG. 55. U. V. SPECTRUM OF DIXANTHENYL DERIVATIVE OF SULFATHIAZOLE (ETHANOL)

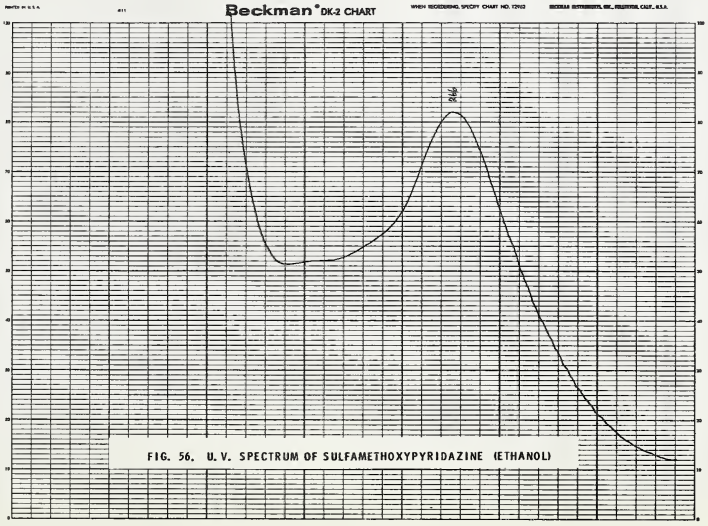


FIG. 56. U. V. SPECTRUM OF SULFAMETHOXYPYRIDAZINE (ETHANOL)

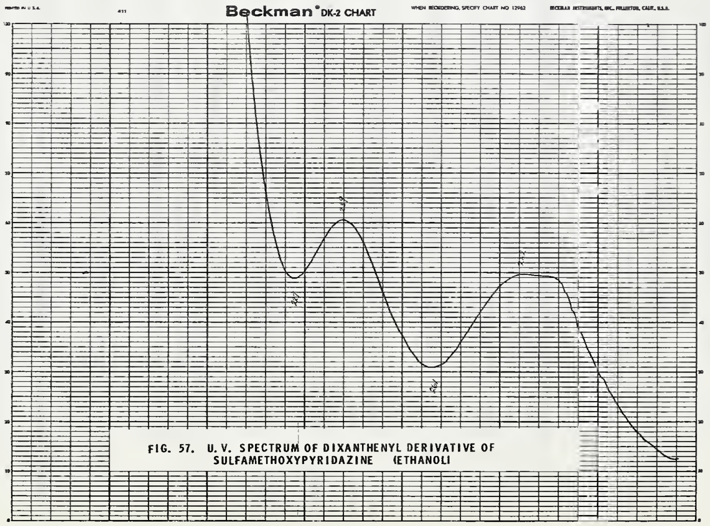
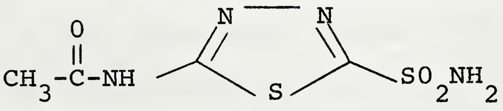
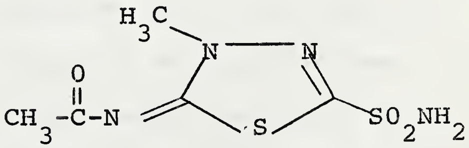


FIG. 57. U. V. SPECTRUM OF DIXANTHENYL DERIVATIVE OF SULFAMETHOXYPYRIDAZINE (ETHANOL)

been employed as a means of distinguishing the amino from the imino tautomeric form (149). These workers found that the above stretching frequency occurred low in the region for sulfanilamides having the imino structure when compared with the corresponding amino tautomers. Accordingly, they assigned the 1170-1145 cm^{-1} and the 1145-1130 cm^{-1} regions as representing the amino and the imino forms respectively. The shift in the position of this absorption was attributed to the difference in the S=0 double bond character between the two forms. An analogous phenomenon has been reported for the carbonyl absorption. Sheinker (147) found that in fixed imino compounds the carbonyl band was observed in the 1630-1605 cm^{-1} region, being substantially shifted from the 1718-1686 cm^{-1} position in the corresponding amino compounds. Corroboration of the above was obtained from a comparison of the carbonyl absorption in acetazolamide (CXCIV) (Figure 58) and methazolamide (CXCV) (Figure 59). The carbonyl band appeared at 1685 cm^{-1} and at 1602 cm^{-1} respectively, showing a very marked shift to lower frequency in the fixed imino structure. An examination of the infrared spectrum of xanthydrol (Figure 52) did not reveal any



CXCIV



CXCV

strong absorptions in the 1170-1130 cm^{-1} region which could complicate interpretation in this region. Accordingly, a comparison was made of the SO_2 symmetric stretching frequencies in the xanthenyl derivatives in an attempt to accomplish a similar distinction. A summary of the positions of these frequencies in the mono and dixanthenyl derivatives, compared with those in the parent compounds, is given in Table VI.

As is apparent from the table, the SO_2 symmetric stretching frequencies appeared in the range of approximately 1160 to 1125 cm^{-1}. Thus, in line with Uno's findings (149), a more appropriate division would be 1160-1145 cm^{-1} and 1145-1125 cm^{-1} according to the amino and imino tautomeric forms respectively. It should be mentioned that the 1145 cm^{-1} position cannot be considered as a sharp line of demarcation between these tautomeric forms, and thus frequencies bordering this point must be interpreted with caution. The value of this division is therefore of a relative nature. The advisability of such a division was substantiated from a comparison of the band positions in some compounds of fixed or known tautomeric forms. Compounds 22 and 23, in Table VI, both have fixed amino structures by virtue of the acetyl substituent in the N^1-position. It will be observed that both the parent compounds and their N^4-xanthenyl derivatives displayed this stretching frequency well within the range attributed to the amino form (1160-1145 cm^{-1}). On the other hand, compounds 9, 12 and 16 which have been shown by other workers to exist in the imino form, exhibited this absorption within the lower range assigned to this tautomeric form (1145-1125 cm^{-1}).

The implications that can be derived from the data contained in Table VI can best be discussed by dividing the compounds

TABLE VI

SO_2 Symmetric Stretching Frequencies of N^1-Mono-Substituted Sulfanilamides and their Xanthenyl Derivaties.

No.	Compound	SO_2 Symm. Stretch. (cm^{-1})		Figure No.
		Parent	Derivative	
1	Sulfacetamide	1148	1147	60
2	Sulfadiazine	1153	1148	10,11
3	Sulfamerazine	1147	1147	12,13
4	Sulfamethazine	1144	1152	14,15
5	Sulfaproxyline	1151	1151	20,21
6	Sulfamethoxazole	1154	1159	24,25
7	Sulfaphenazole	1148	1155	26,27
8	Sulfadimethoxine	1142	1152	44,45
9	Sulfapyridine	1122	1127	16,17
10	Sulfisomidine	1126	1115	18,19
11	Sulfaguanidine	1127	1133[a]	22,23
12	Sulfamethizole	1126[b]	1139[c]	31,32
13	Sulfaethylthiadiazole	1140	1140	34,35
14	Dibromosulfaethylthiadiazole	1143	1142	46,47
15	Isobuzole	1159[d]	1145	61,62
16	Sulfathiazole	1134	1135	36,37
17	Succinylsulfathiazole	1142	1140	63,64
18	p-Nitrosulfathiazole	1152	1146	65,66
19	Sulfamethoxypyridazine	1153[e]	1133	38,39
20	Sulfachloropyridazine	1144	1134	40,41
21	Sulfisoxazole	1159	1147[f]	42,43
22	N^1-Acetylsulfamethoxypryidazine	1155	1151	48,49
23	N^1-Acetylsulfisoxazole	1156	1160	50,51

a. 1119 cm^{-1} in dixanthenyl derivative (Fig. 28)
b. Shoulder at 1143 cm^{-1}.
c. 1135 cm^{-1} chloroform (Fig. 33).
d. Shoulder at 1139 cm^{-1}.
e. Weak band at 1126 cm^{-1}.
f. Weak band at 1159 cm^{-1}.

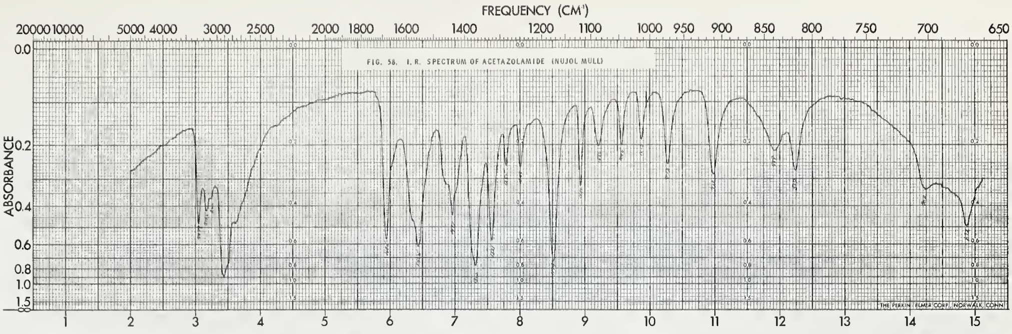


FIG. 58. I. R. SPECTRUM OF ACETAZOLAMIDE (NUJOL MULL)

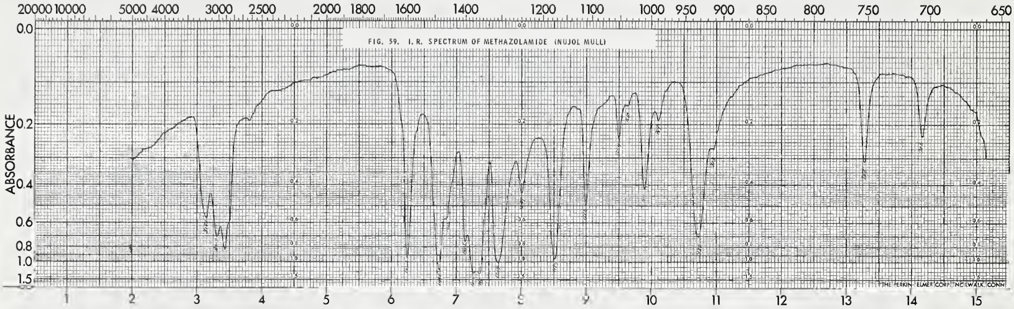


FIG. 59. I. R. SPECTRUM OF METHAZOLAMIDE (NUJOL MULL)

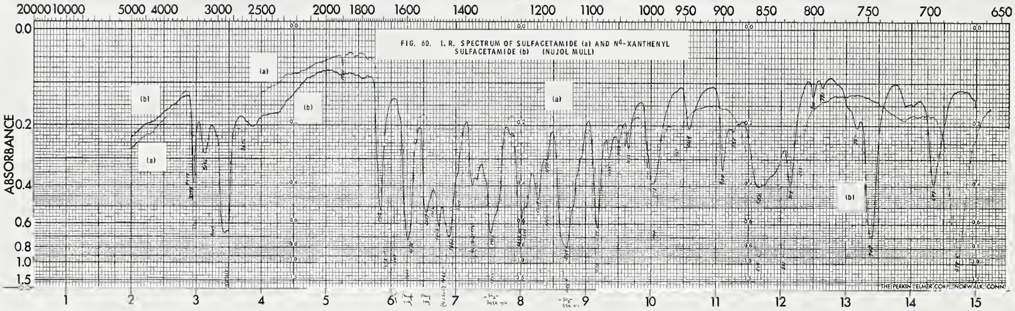


FIG. 60. I. R. SPECTRUM OF SULFACETAMIDE (a) AND N⁴-XANTHENYL SULFACETAMIDE (b) (NUJOL MULL)

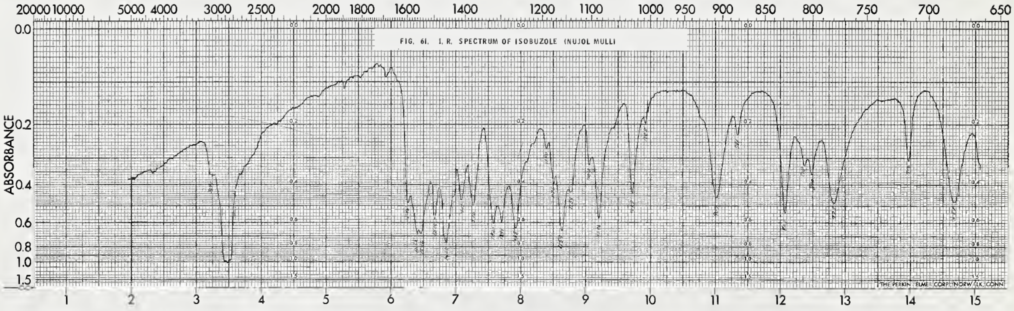


FIG. 61. I. R. SPECTRUM OF ISOBUZOLE (NUJOL MULL)

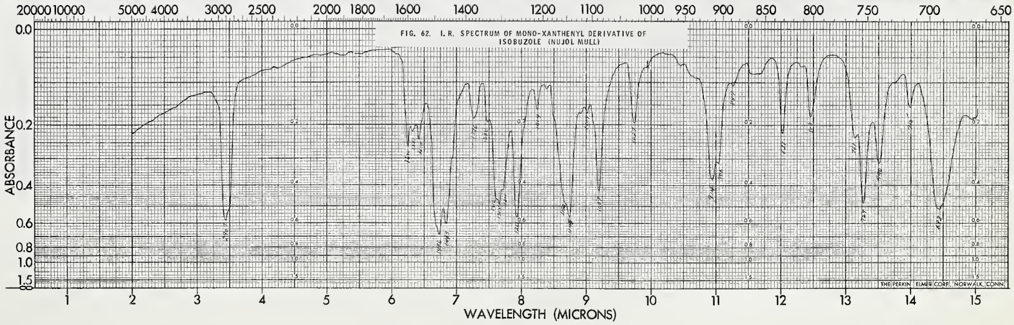


FIG. 62. I. R. SPECTRUM OF MONO-XANTHENYL DERIVATIVE OF ISOBUZOLE (NUJOL MULL)

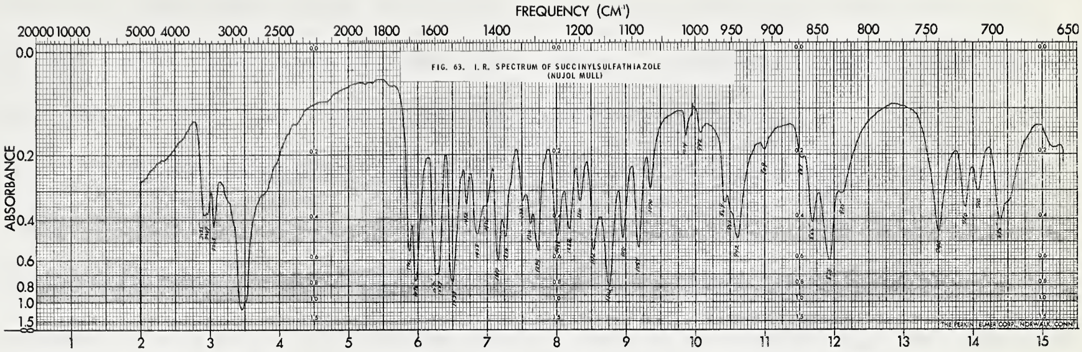


FIG. 63. I.R. SPECTRUM OF SUCCINYLSULFATHIAZOLE (NUJOL MULL)

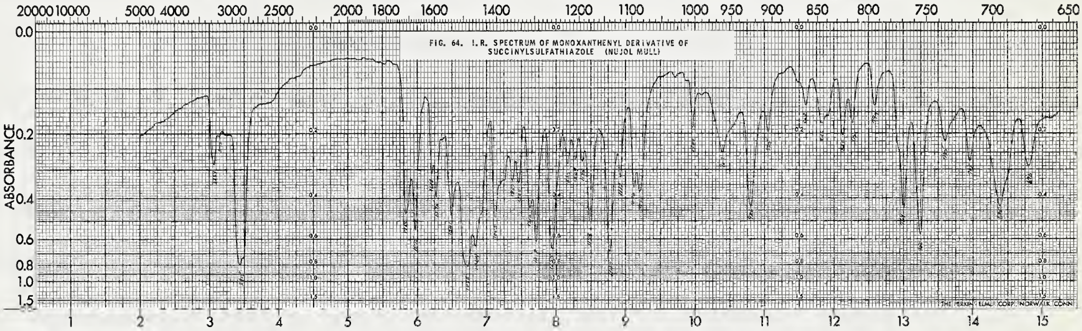


FIG. 64. I.R. SPECTRUM OF MONOXANTHENYL DERIVATIVE OF SUCCINYLSULFATHIAZOLE (NUJOL MULL)

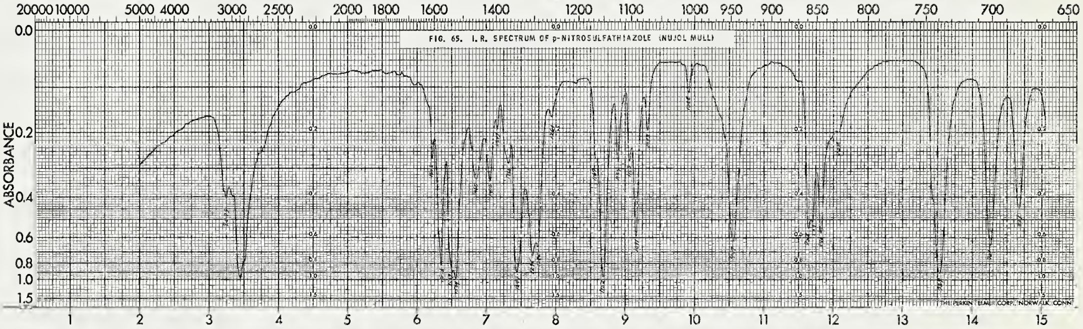


FIG. 65. I.R. SPECTRUM OF p-NITROSULFATHIAZOLE (NUJOL MULL)

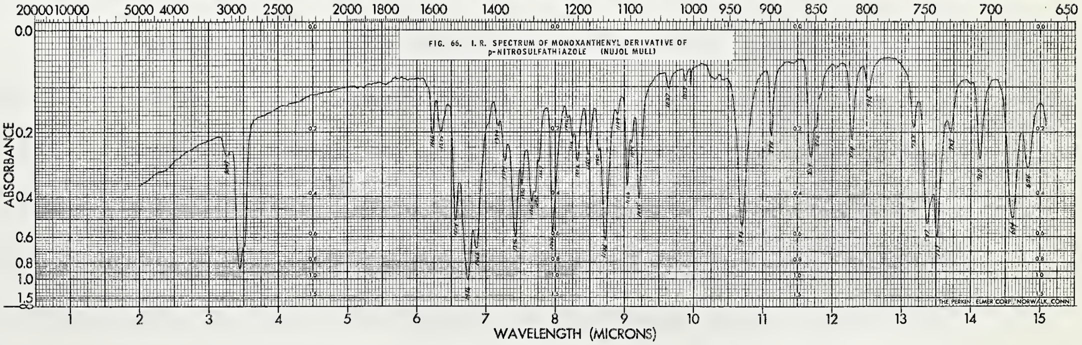
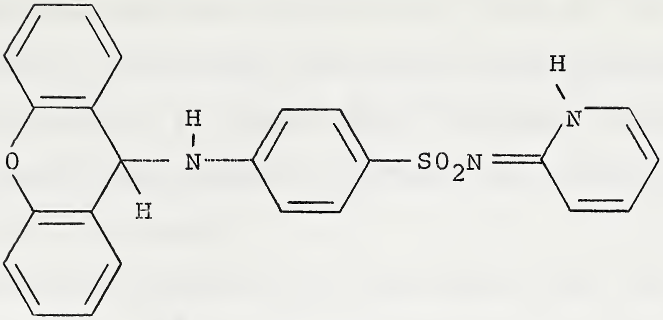


FIG. 66. I.R. SPECTRUM OF MONOXANTHENYL DERIVATIVE OF p-NITROSULFATHIAZOLE (NUJOL MULL)

into two groups. The first of these represents those compounds in which the hydrogen atom involved in the prototropic tautomerism has not been substituted, and the second those in which the reaction with xanthydrol has concerned this hydrogen atom.

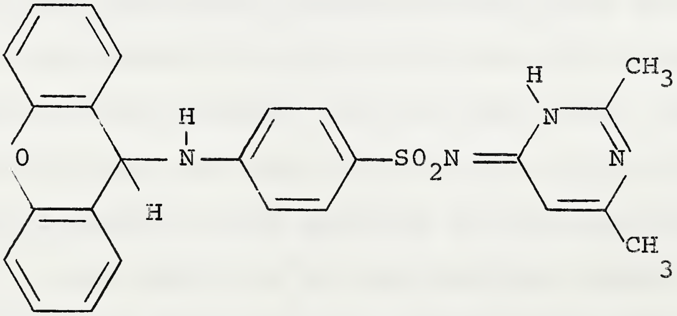
The sulfanilamides falling in this first category are those listed as numbers 1 to 11, inclusive, in Table VI. It will be recalled that the xanthenyl derivatives in this group, with the exception of that of sulfaguanidine (No. 11), could all be titrated as acids, thereby establishing the free nature of the hydrogen atom concerned in the prototropic tautomerism. Considering only the first 8 sulfanilamides, for the moment, it will be seen that in general the frequencies occurred in the amino region (1160-1145 cm^{-1}) for both the parent compounds and their derivatives. In compounds 4 and 8 the absorptions fell somewhat short of the lower boundary, but in both instances these were shifted to 1152 cm^{-1} in their xanthenyl derivatives. Whether the above two parent compounds actually exist in the imino form cannot be deduced from this information alone. Nevertheless, it would appear that the xanthenyl derivatives of the first 8 compounds possess the amino tautomeric form.

The succeeding three compounds constitute exceptions to the preceding observation. Sulfapyridine (No.9), as mentioned earlier, has been shown to exist in the imino form by several authors (147, 148,149). The appearance of the SO_2 band at nearly the same position in its N^4-xanthenyl derivative suggests that the structure of this derivative is probably most accurately described by its imino tautomer (CXCVI).



CXCVI

Although there has not been any work reported concerning the favored tautomeric form of sulfisomidine (No.10), the relatively low SO_2 frequencies in both the parent (1126 cm^{-1}) and its N^4-xanthenyl derivative (1115 cm^{-1}) strongly suggests the imino form in both instances. Accordingly, structure CXCVII probably more accurately describes this derivative than would its amino isomer.



CXCVII

Sulfaguanidine (No. 11), it will be recalled, afforded a monoxanthenyl derivative as the major product and a small amount of a dixanthenyl product. The evidence regarding sites of substitution was, however, inconclusive. The low frequencies of the SO_2

symmetric stretching bands in the parent compound and in its mono and dixanthenyl derivatives, similarly infer an imino structure in each instance. By analogy with other sulfanilamides, the mono-substituted product is likely the N^4-xanthenyl derivative, whereas the dixanthenyl derivative is probably also substituted on one of the guanidino nitrogens.

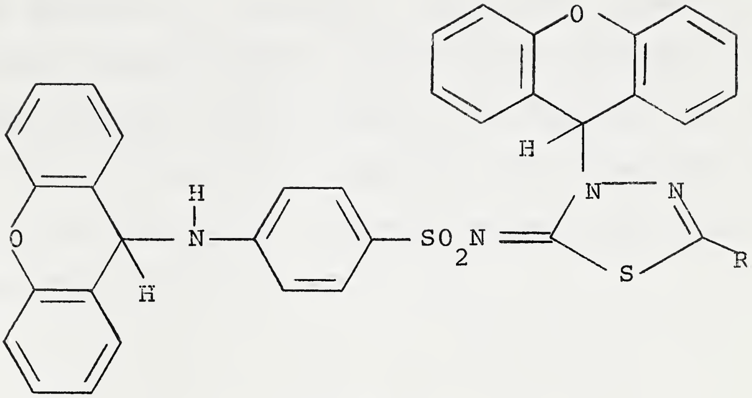
It should be mentioned at this point that the imino structures postulated for the N^4-xanthenyl derivatives of sulfapyridine (CXCVI) and sulfisomidine (CXCVII) refer only to the solid state. When dissolved in N,N-dimethylformamide (DMF), these must exist wholly or in part in the amino form in order to account for their titratability.

The second group constitutes those sulfanilamides designated as numbers 12 to 21, inclusive, in Table VI. The absence of an acidic hydrogen at N^1 was demonstrated for these derivatives from their failure to titrate as acids. It will be seen that the frequencies for the parent compounds range from 1159 to 1126 cm^{-1}, suggesting the presence of both amino and imino tautomeric forms among these sulfanilamides. On the other hand, the range in frequencies for the xanthenyl derivatives (1147-1133 cm^{-1}) corresponds closely to that assigned to the imino form (1145-1125 cm^{-1}). This reduction in the frequency range can be attributed to the substantial shift to lower frequencies, apparent in several compounds, upon going from the parent compound to the derivative. The remainder of the sulfanilamides in this category displayed a relative constancy in their SO_2 symmetric stretching frequencies. These observations strongly suggest that the reaction with xanthydrol at this site resulted in a substitution of

the N^1 hydrogen atom in the sulfanilamides from the imino tautomeric form. They imply further that the parent compounds need not necessarily exist as the imino tautomers in order to obtain substitution from this form. This would suggest that these sulfanilamides exist in a tautomeric equilibrium in glacial acetic acid, the reaction medium employed, and that they condense with xanthydrol from the imino form.

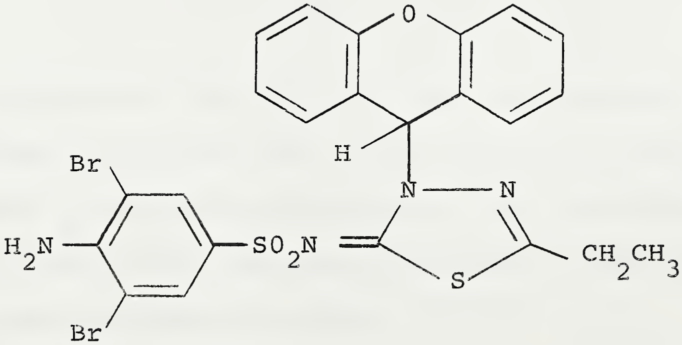
Sulfamethizole (No. 12), as mentioned earlier, has been shown by Sheinker et al.(150) to exist in the imino tautomeric form. Its spectrum (Figure 31) showed a strong SO_2 stretching absorption at 1126 cm^{-1} with a well defined shoulder at 1143 cm^{-1}. This doublet became a sharp single band, appearing at 1139 cm^{-1}, in the derivative (Figure 32). As this latter frequency is still well within the imino region, the following structure CXCVIII can be assigned to its dixanthenyl derivative. On the basis of the close proximity of this absorption in the ethyl homolog (No. 13) and its dixanthenyl derivative, these would also appear to exist in the imino form. Accordingly, a comparable structure may be assigned to the dixanthenyl derivative of sulfaethylthiadiazole (CXCIX).

The dibromo analog of sulfaethylthiadiazole (No. 14) also displayed its SO_2 frequency at nearly the identical position, and hence it too would be expected to exist in the imino form. Since no shift was observed in its monoxanthenyl derivative, the imino structure may also be ascribed to this compound (CC).



CXCVIII, R = CH_3

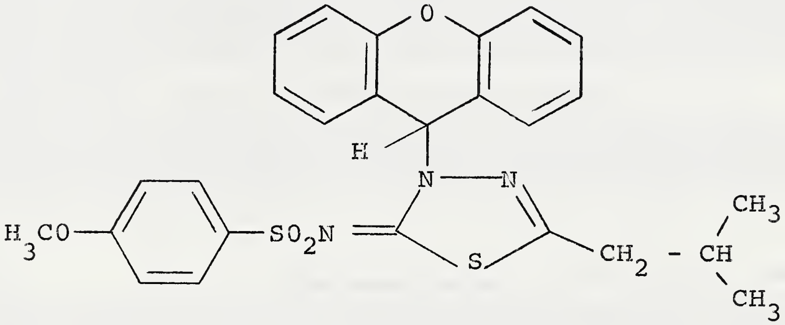
CXCIX, R = C_2H_5



CC

The results with isobuzole (No. 15) are somewhat ill-defined. Although the 1159 cm^{-1} band has been attributed to the SO_2 symmetric stretching frequency, it may well be that the band at

1139 cm^{-1}, appearing as a shoulder on the former band, should have been accorded this assignment. In support of the above, the ultraviolet spectrum of isobuzole (Figure 67) showed two well defined maxima characteristic of the imino tautomeric form. In view of these results, it is difficult to identify the favored tautomeric form in the parent compound. The relatively low frequency in the derivative, however, suggests the imino form for its xanthenyl derivative (CCI).



CCI

Sulfathiazole (No. 16), it will be recalled, has also been demonstrated to possess the imino structure (147,148,149,150). Since the SO_2 stretching frequency in its dixanthenyl derivative appears at nearly the identical position, it may therefore be assigned the following structure (CCII):

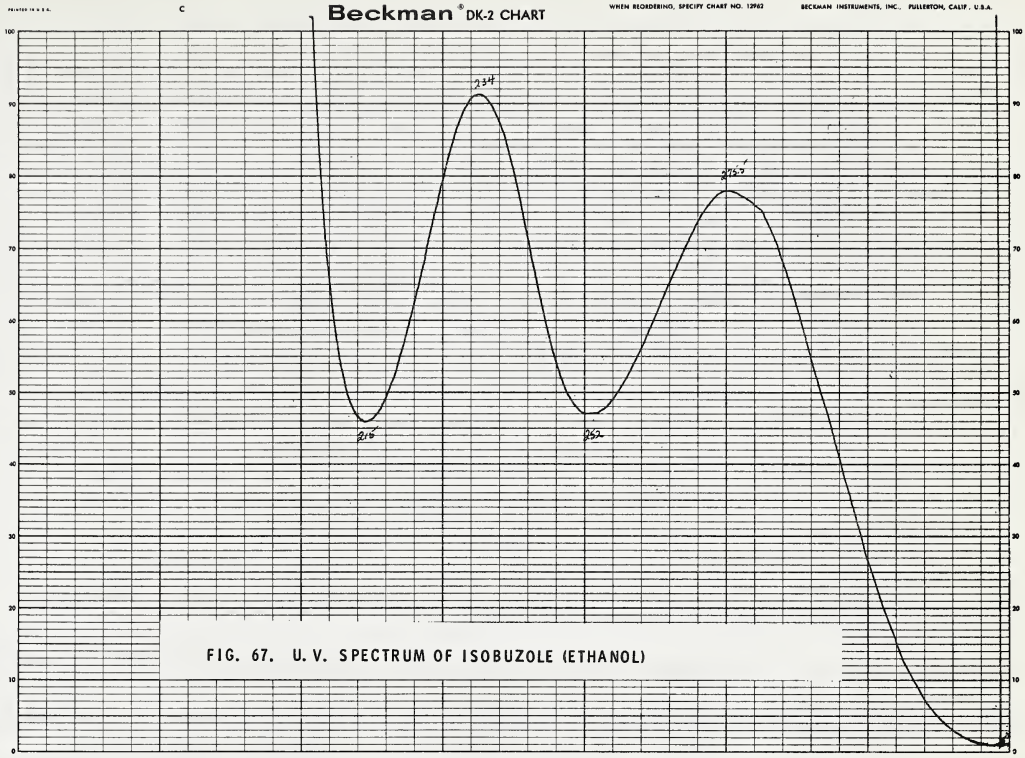


FIG. 67. U. V. SPECTRUM OF ISOBUZOLE (ETHANOL)

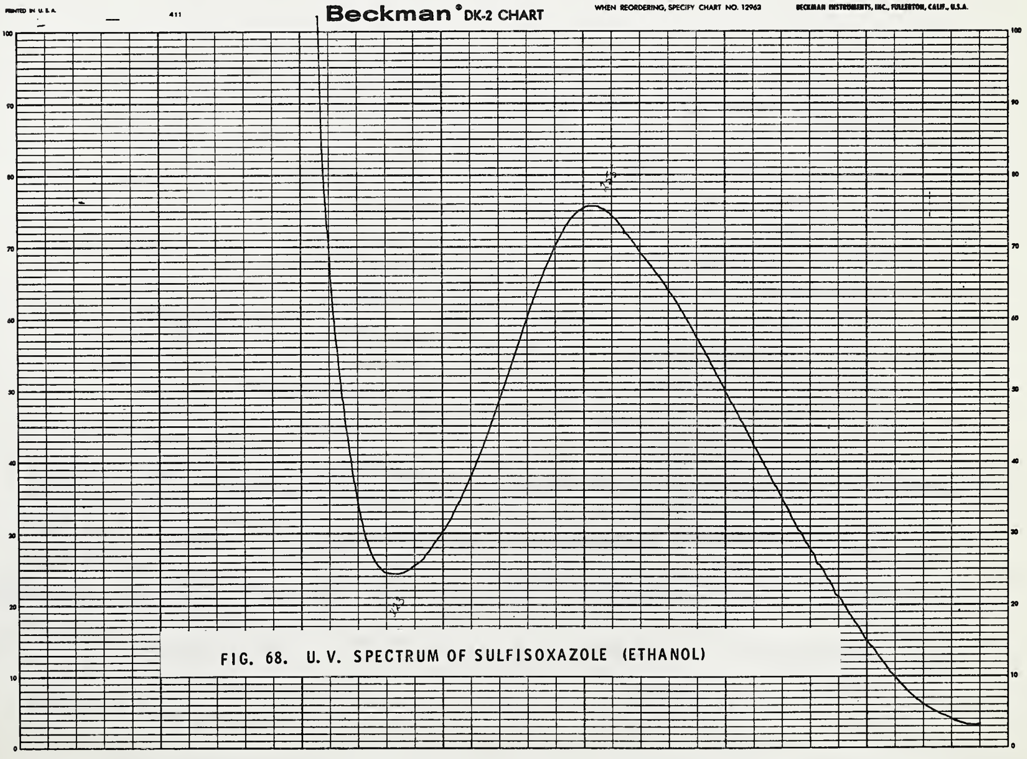
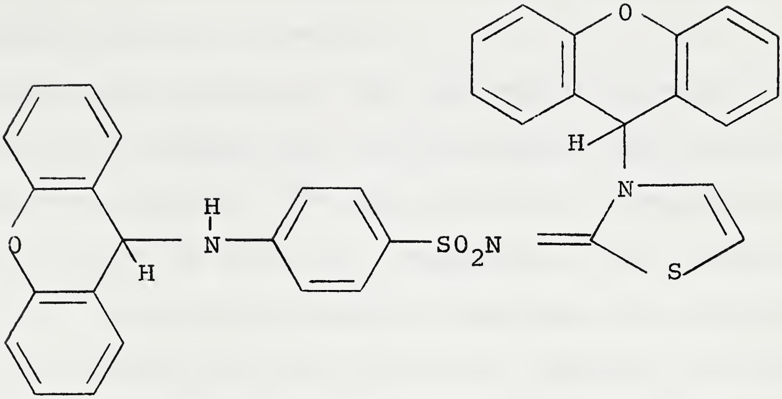
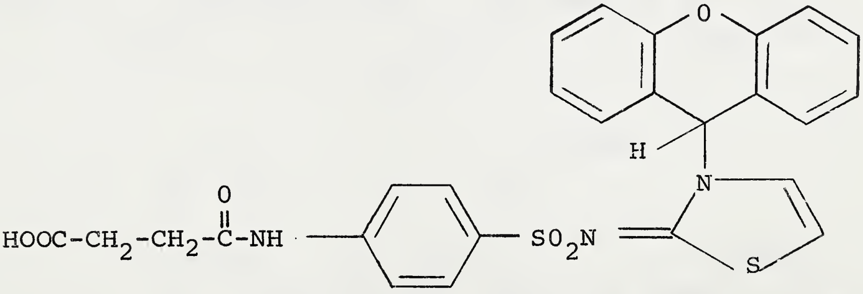


FIG. 68. U. V. SPECTRUM OF SULFISOXAZOLE (ETHANOL)



CCII

The N^4-succinyl analog of sulfathiazole (No. 17) and its xanthenyl derivative also displayed this frequency in the imino region and consequently a comparable structure may be assigned to this derivative (CCIII)



CCIII

p-Nitrosulfathiazole (No.18) showed its stretching frequency at 1152 cm^{-1}, implying the amino tautomeric form for the parent compound. A small shift to 1146 cm^{-1} occurred in its xanthenyl derivative presenting a difficult situation to interpret. By

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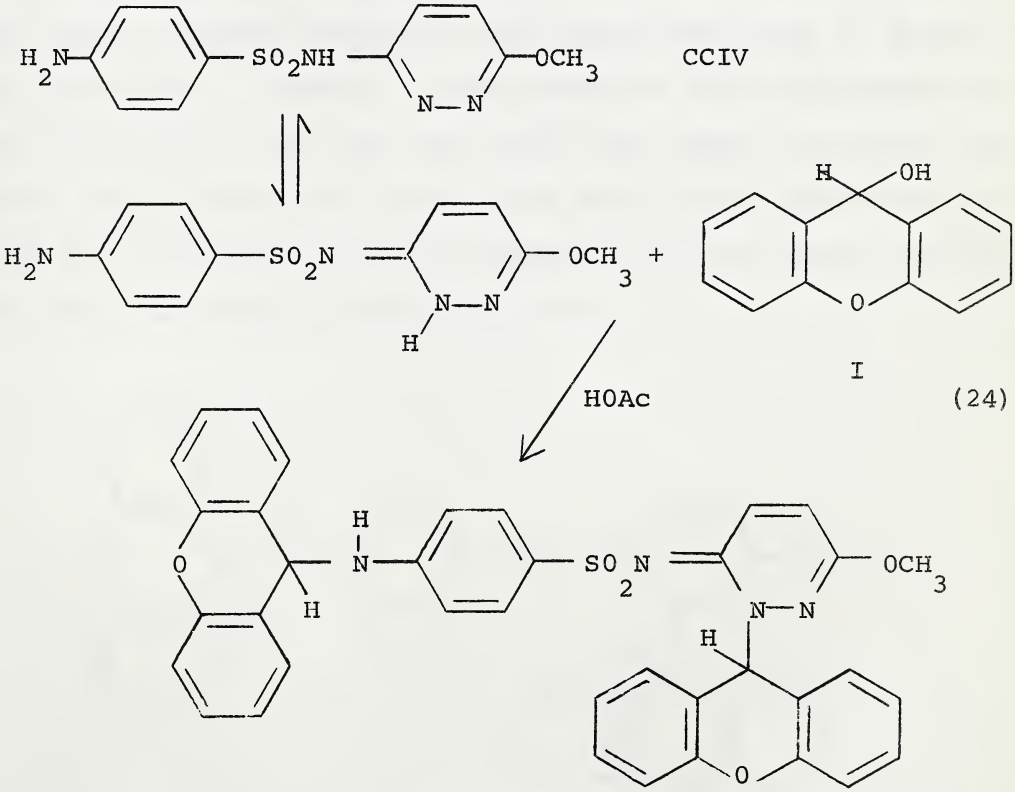
analogy with the other members in the sulfathiazole series the imino form for this xanthenyl derivative would appear to be the most likely structure as well.

Sulfamethoxypyridazine (No. 19), exhibited quite a substantial shift to lower frequency in its dixanthenyl derivative as compared to the parent compound. The SO_2 stretching frequency in the parent compound occurred at 1153 cm^{-1}, although it too showed a weak band at 1126 cm^{-1} which could possibly represent this absorption. The former band implies an amino structure, whereas the latter would be typical of the imino isomer. As the ultraviolet spectrum of the parent compound (Figure 56) displayed only a single maximum,



CCIV

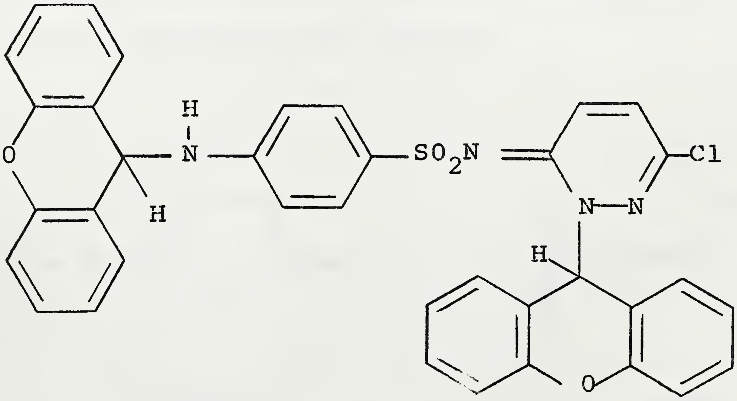
I

(24)

CCV

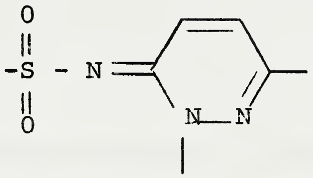
exemplary of the amino form, the 1153 cm^{-1} assignment would appear to be correct. Consequently, this compound probably assumes the amino tautomeric form in the solid state. The SO_2 frequency in its dixanthenyl derivative is observed at 1133 cm^{-1}, a shift of 20 cm^{-1}, well within the imino range. It thus appears that sulfamethoxypyridazine (CCIV) exists in tautomeric equilibrium, in glacial acetic acid, and that condensation with xanthydrol (I) occurs from the imino form to yield the resulting dixanthenyl derivative (CCV) (equation 24).

Sulfachloropyridazine (No. 20) similarly showed a shift to lower frequency in its derivative as compared to the parent compound. The frequency of the SO_2 stretching band occurred at 1144 cm^{-1} in the parent compound suggesting the possibility that it exists in the imino form. However, it too shows but a single maximum in the ultraviolet (154) and thus would also appear to assume the amino form in the solid state. The shift in the derivative to 1134 cm^{-1} implies a structure comparable to its methoxy analog for this dixanthenyl derivative (CCVI).



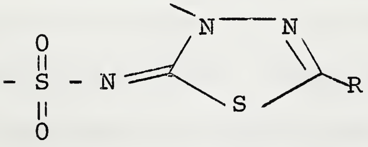
CCVI

The apparent change in tautomeric forms accompanying the reactions of the above two compounds may serve to explain the colored compounds recovered in both instances. Sulfamethoxypyridazine, which is white in color, yielded a yellow dixanthenyl derivative. The chloro analog, a pale yellow in the pure state, provided a bright yellow derivative. All other sulfanilamides showed no change in color upon derivatization. This color in the derivatives is due, undoubtedly, to the conjugated system established in these molecules (CCVII), which is not present in the amino tautomeric forms of the parent compounds.

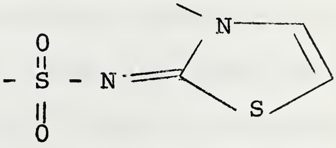


CCVII

The previous derivatives of the thiadiazole (CCVIII) and thiazole (CCIX) series did not create a similar conjugated system and thus would not be expected to be colored.

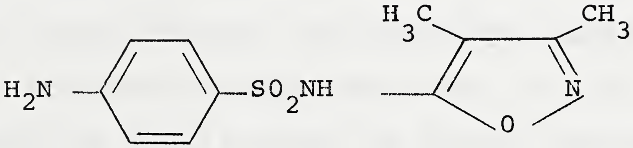


CCVIII



CCIX

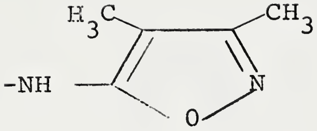
The last compound in this category, namely sulfisoxazole (No. 21), appears to represent a unique situation not previously encountered in this study. The SO_2 symmetric stretching frequency at 1159 cm^{-1}, coupled with only one maximum in the ultraviolet (Figure 68) suggests strongly that the parent compound assumes the amino form (CCX) in the solid state.



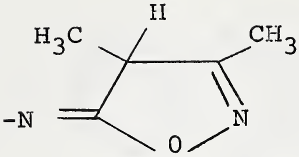
CCX

An apparent shift to lower frequency (1147 cm^{-1}) occurred in its derivative, although a weak, clearly defined band also appeared at the identical 1159 cm^{-1} position. Assuming the stronger former band to be the true SO_2 symmetric stretching frequency in the derivative still poses a problem in interpretation because of its borderline position.

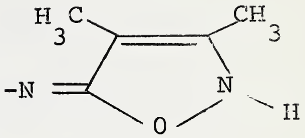
Prototropic tautomerism in this sulfanilamide is of a slightly different nature, from those previously encountered, as the annular nitrogen atom is now considerably removed from the extra-ring nitrogen. Three tautomeric forms are possible in this compound, as represented below (CCXI, CCXII and CCXIII). Structure CCXII is the least likely possibility, for the reactive form, because of the steric inaccessibility at this site. Reaction from the latter (CCXIII) tautomeric form would establish



CCXI



CCXII



CCXIII

a conjugated system, similar to that encountered with the pyridazine sulfanilamides, and accordingly would be expected to yield a colored xanthenyl derivative. In fact, however, no color change was found between the parent compound and the derivative. In view of the above, reaction would appear to have occurred from the amino (CCXI) tautomeric form. Several reports in the literature tend to substantiate this conclusion.

Boulton and Katritzky (155), from infrared and nuclear magnetic resonance studies, found the 5-amino-as well as 5-acetamido-isoxazoles existed predominantly in the amino form CCXI. Enoki (156), from a study on the acetylation of sulfanilamides, found that sulfisoxazole (CCX) as opposed to other sulfanilamides readily underwent selective acetylation at the N^1-position. He attributed the exceptional reactivity at this site to the greater relative acidity of this sulfanilamide.

On the basis of the above observations, it would appear that sulfisoxazole has in fact condensed with xanthydrol from its amino form. Accordingly, the dixanthenyl derivative of sulfisoxazole may be assigned the following structure (CCXIV):

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[illegible]

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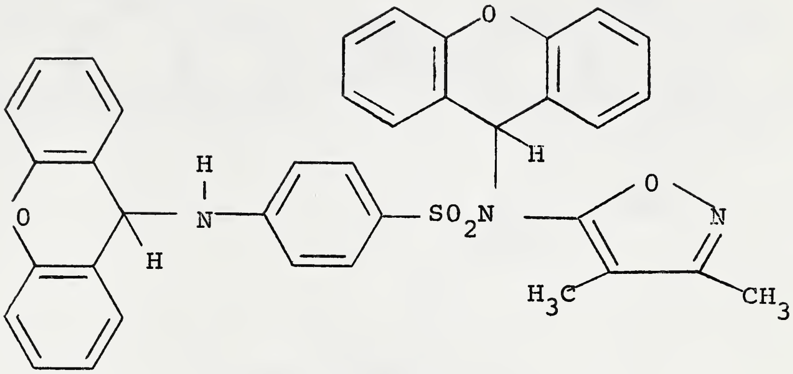

CCXIV

The above compound represents the fourth distinct site of substitution encountered in this study. The diversified type of substitution once again serves to illustrate the great reactivity of xanthydrol.

4. Reactivity of Xanthydrol

The exceptional reactivity of xanthydrol, as opposed to other secondary alcohols, has often been acknowledged but has never been satisfactorily explained.

Reactions with xanthydrol (I) involve the xanthylium ion (CCXV), which is apparently readily formed in glacial acetic acid. Electrophilic attack of an electron pair from a molecule of relatively high electron density leads to the substitution product (CCXVI) (equation 25) (5,157). Mizukami and Kanaya (42) investigated the mechanism of this reaction with phenol and found that it followed second order reaction kinetics. This is, however,

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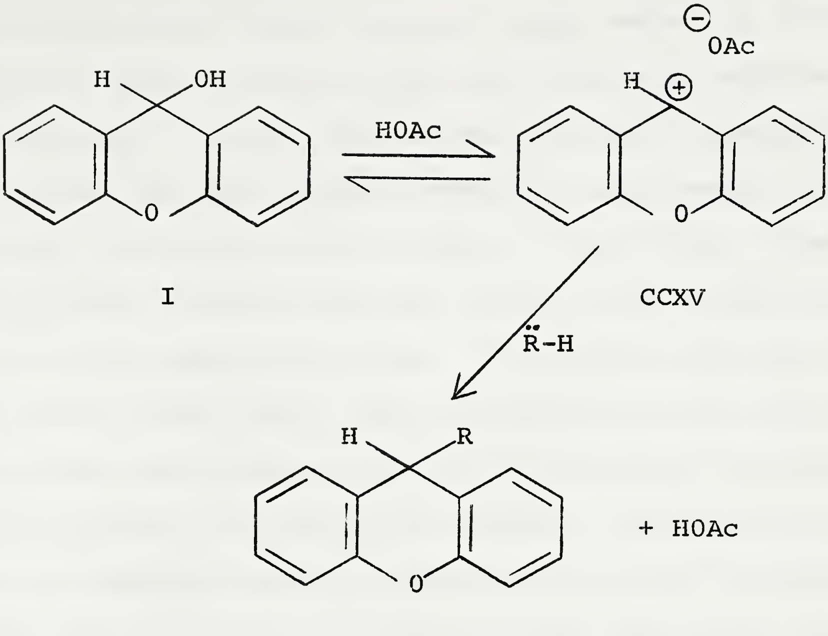


I CCXV CCXVI (25)

the only mechanistic study with xanthydrol contained in the literature.

The ability of xanthydrol to yield the carbonium ion (CCXV), in such a weak acid, indicates that the hydroxyl group in the former must be quite basic. The ease of formation of a carbonium ion from a carbinol in acidic medium has been considered to reflect what has been termed the " secondary basicity" (158) of the carbinol (159). The expression of this basicity in terms of values of pK_{R^+}* provides a basis for comparison of carbinols.

From a spectroscopic study of the equilibrium constant (K_{R^+}) in the reaction

$$R^{\oplus} + HOH \rightleftharpoons ROH + H^{\oplus},$$

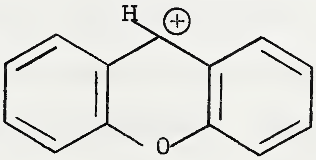
---

* the negative logarithm of the equilibrium constant for the reaction.

Arnett and Bushick (160) obtained a pK_{R^+} value for xanthydrol of -0.17. Deno and Evans (161) had previously reported a value of $pK_{R^+} = -0.84$. The secondary alcohol benzhydrol, by comparison, has been found to exhibit a much weaker "secondary basicity" yielding a value of $pK_{R^+} = -13.3$ (162). Even the tertiary alcohol triphenylcarbinol ($pK_{R^+} = -6.44$ (160)), has been shown to be a much weaker base. The latter value has also been reported as -6.63 (162). This comparison may be carried further, and in fact both Deno et al. (162), and Arnett and Bushick (160), found xanthydrol to display the highest "secondary basicity" of all the carbinols studied, exclusive of 4,4',4"-trimethoxytriphenyl carbinol ($pK_{R^+} = + 0.82$). It has been shown that triphenylcarbinol is sufficiently less basic than xanthydrol, that carbonium ion formation in glacial acetic acid for the former is negligible and results only after the addition of sulfuric acid (158).

The ease of formation of the xanthylium ion (CCXV) suggests the likelihood of an inherent stability being associated with this ion. A comparison of the resonance forms for the carbonium ions resulting from xanthydrol, benzhydrol and triphenylcarbinol revealed the following: nine canonical structures can be drawn for xanthydrol, seven for benzhydrol and ten for triphenylcarbinol. However, considering the relative contributions of these resonance forms, one must take into account the very significant contributions of the two oxonium ion structures to the stability of the xanthylium ion. Similar important contributors are not present in the carbonium ions of benzhydrol or triphenylcarbinol.

A Fisher-Hirschfelder-Taylor model of xanthydrol revealed this structure to be buckled, by virtue of the tetrahedral carbon atom in the 9-position. The xanthylium ion (CCXV), on the other hand, proved to be a planar structure, due to the trigonal nature of the carbon atom at this position. Similar studies with a model of the triphenylcarbinol carbonium ion revealed that all of the benzene rings could not be made to lie in a common plane. Such an arrangement must involve a loss of resonance stabilization (159), since maximum resonance stability is commonly associated with planarity. A closer examination of the xanthylium ion (CCXV) disclosed that the molecule fulfilled all the requirements for aromaticity. It is planar, stabilized by resonance, and has 14π electrons thereby satisfying the Hückel rule for aromaticity. This carbonium ion (CCXV) may be considered as being isoelectric with anthracene (163). It is our contention, therefore, that the exceptional ease of formation of this carbonium ion is associated with the gain in stability accompanying the transformation, due to the aromatic character of the resulting ion.



CCXV

The great stability of this carbonium ion was demonstrated by storing a solution of xanthydrol in glacial acetic acid for two months prior to its use in a condensation reaction. Equally good results were obtained from this solution as compared to a freshly prepared one. Phillips and Pitt (4), for convenience sake,

employed a stock solution of xanthydrol in acetic acid for preparing derivatives of primary amides. They reported no decomposition after periods as long as four months and actually considered this reagent to be more stable in this form than in the dry state.

The exceptional ease of formation of the xanthylium ion was demonstrated as a result of an observation made early in our investigation. While attempting to uncover a suitable method for preparing the xanthenyl derivatives, an excellent yield of N^4-xanthenyl-sulfacetamide was obtained from the condensation of xanthydrol with sulfacetamide in 95% ethanol. This product had separated before any acetic acid had been added to the reaction mixture. The above procedure was readily duplicated. Thus, it was obvious that the xanthylium ion had been formed in ethanol without any acetic acid being present. A comparison of the pKa's of acetic acid (4.76) and sulfacetamide (5.38) (131) suggested that perhaps even this weaker acid, acting as its own catalyst, was capable of yielding the xanthylium ion. Verification of this hypothesis was obtained from the results of several other condensations. Sulfamethizole (pKa = 5.45 (131), when condensed with xanthydrol in ethanol, likewise afforded a dixanthenyl derivative identical with that obtained previously by the general procedure. Similarly, p-amino benzoic acid (pKa = 4.68 (131)) when condensed with xanthydrol in ethanol yielded a xanthenyl derivative identical with that prepared in acetic acid. The considerably weaker acid sulfapyridine (pKa = 8.43 (131)), on the other hand, failed to react in ethanol yielding only unreacted starting material. The weakly basic urea,

yielded no product after a month, but on the addition of acetic acid, dixanthenyl urea separated in a matter of 30 minutes. The hypothesis was further substantiated by demonstrating the necessity of an ionizing solvent. Xanthydrol and sulfacetamide allowed to stand in acetone for a period of a month failed to yield a product. Upon addition of 95% ethanol to the mixture, the xanthenyl derivative separated after several days.

The preceding results suggest that acids even weaker than glacial acetic are capable of protonating the hydroxyl group in xanthydrol and thus yielding the corresponding xanthylium ion. Furthermore, with compounds possessing pKa's in the vicinity of 5.5, and being otherwise capable of condensing with xanthydrol, the reactions in ethanol are autocatalytic and require no outside acid catalyst. This exceptional reactivity must therefore be associated with the inimitable stability of the xanthylium ion, which in turn must be related to the aromatic character of this carbonium ion.

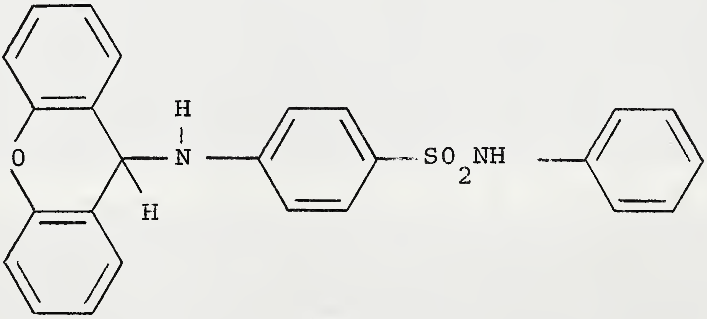
5. Applications of this Reaction

The value of xanthydrol as a reagent for the characterization of sulfanilamides is somewhat hampered by the instability of the resulting derivatives. Nevertheless, the procedure is applicable for all therapeutically important sulfanilamides and thus may perhaps serve a useful purpose in this capacity.

Due to the nature of the procedure adopted for the preparation of these derivatives, the method is directly applicable to commercial preparations of these sulfanilamides. With tablets, for example, simple extraction of the sulfanilamide with N,N-dimethyl-

formamide (DMF) provides the basis for the succeeding step in the method. This is analogous to the direct applicability to tablets reported for the nonaqueous titration of sulfanilamides in DMF (130). A single trial on a commercial preparation (Sulamyd Tablets - Schering) revealed that the procedure was indeed applicable.

One other N^1-monosubstituted sulfanilamide, not employed therapeutically, also afforded a monoxanthenyl derivative. Sulfanilanilide (N^1-phenyl-sulfanilamide) yielded a product which, as expected, exhibited the characteristic signs of instability common to these compounds. The derivative was titratable as an acid, and its infrared spectrum displayed only a single band at 3317 cm^{-1} (Figure 69) as opposed to bands at 3367 and 3306 cm^{-1} in the parent compound (Figure 70). The NH_2 deformation band at 1641 cm^{-1} had also disappeared in the derivative. Accordingly, the derivative must be N^4-xanthenyl-N^1-phenyl-sulfanilamide (CCXVII).



CCXVII

A reaction with an N^1-monosubstituted metanilamide revealed a similar site of substitution in these position isomers. Metachloridine (N^1-(5-chloro-2-pyrimidyl)-metanilamide), when condensed with xanthydrol, afforded what proved to be the corresponding N^3-xanthenyl derivative (CCXVIII).

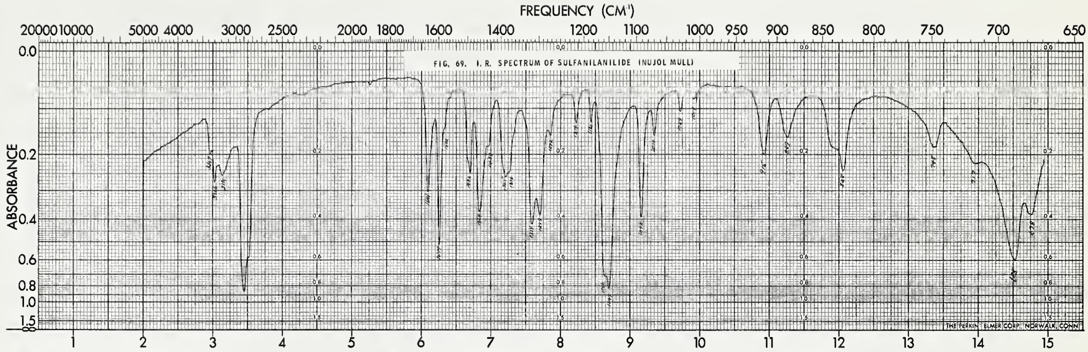


FIG. 69. I. R. SPECTRUM OF SULFANILANILIDE (NUJOL MULL)

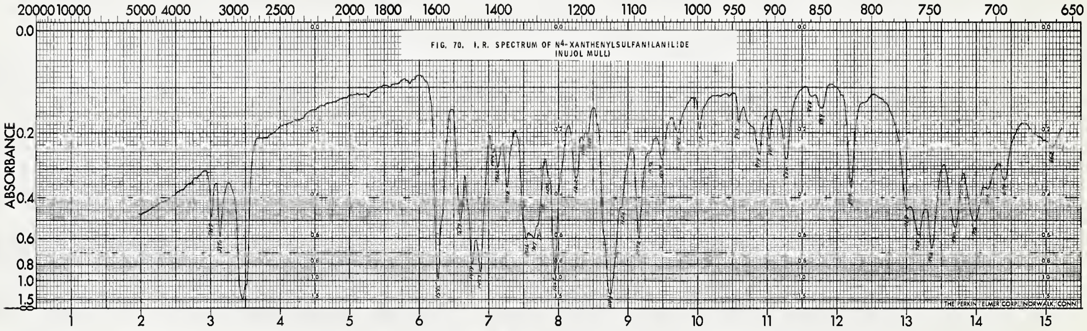


FIG. 70. I. R. SPECTRUM OF N^4-XANTHENYLSULFANILANILIDE (NUJOL MULL)

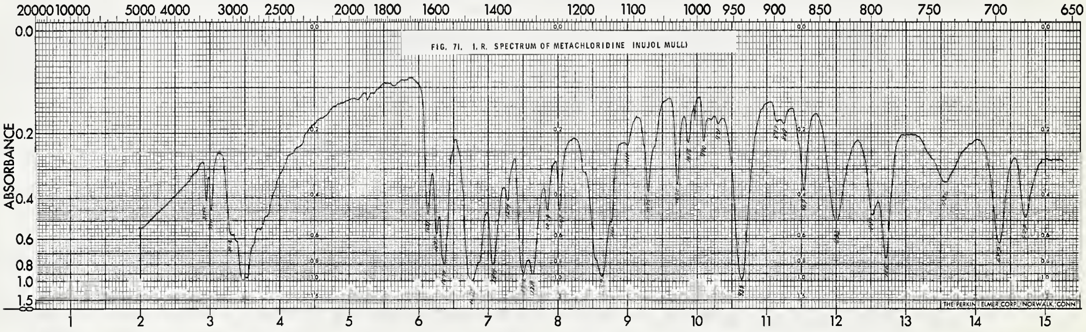


FIG. 71. I. R. SPECTRUM OF METACHLORIDINE (NUJOL MULL)

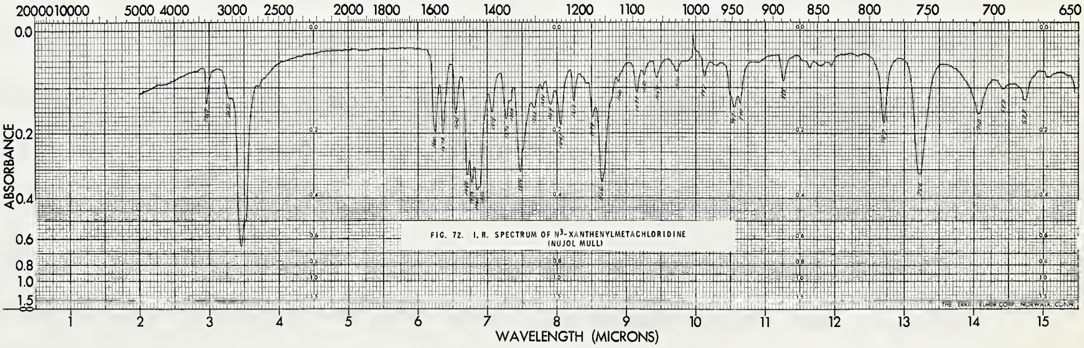
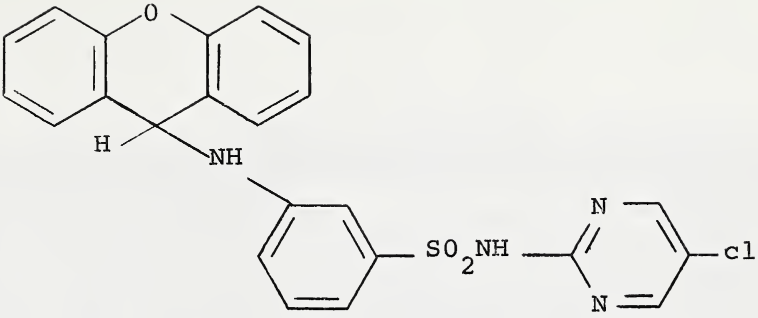


FIG. 72. I. R. SPECTRUM OF N^3-XANTHENYLMETACHLORIDINE (NUJOL MULL)



CCXVIII

This compound was found to be even more unstable than the N^4-xanthenyl derivatives of the sulfanilamides. The crude product was cream in color, very unstable toward recrystallization, but white in color when finally obtained in a pure form. However, on standing at room temperature in the dry state the product acquired a bright orange color, a phenomenon not encountered with the sulfanilamides. The purified derivative yielded a quantitative recovery upon titration in nonaqueous media. Proof for the above structure (CCXVIII) was again obtained from an examination of its infrared spectrum. The parent compound showed NH stretching vibrations at 3390 and 3311 cm^{-1} and an NH deformation vibration at 1631 cm^{-1} (Figure 71). The derivative, on the other hand, showed only a single absorption in the former region (3367 cm^{-1}) and the deformation band had disappeared (Figure 72).

The success of this reaction with N^1-monosubstituted sulfanilamides, in general, and with p-aminobenzoic acid, in particular, suggested its application to local anesthetics of the p-aminobenzoate type. Condensation of ethyl p-amino benzoate (benzocaine) with xanthydrol yielded a monoxanthenyl derivative which was also

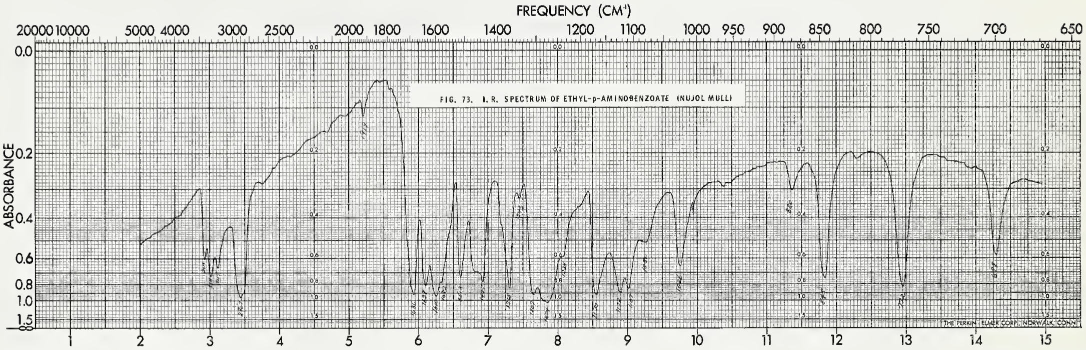


FIG. 73. I. R. SPECTRUM OF ETHYL-p-AMINOBENZOATE (NUJOL MULL)

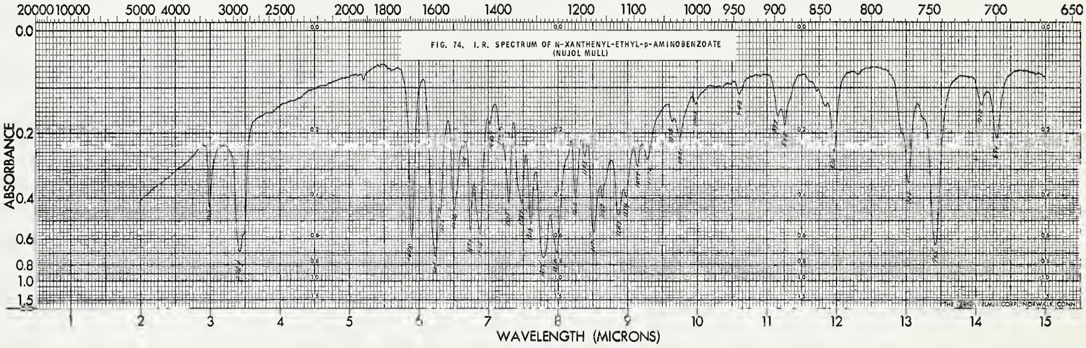


FIG. 74. I. R. SPECTRUM OF N-XANTHENYL-ETHYL-p-AMINOBENZOATE (NUJOL MULL)

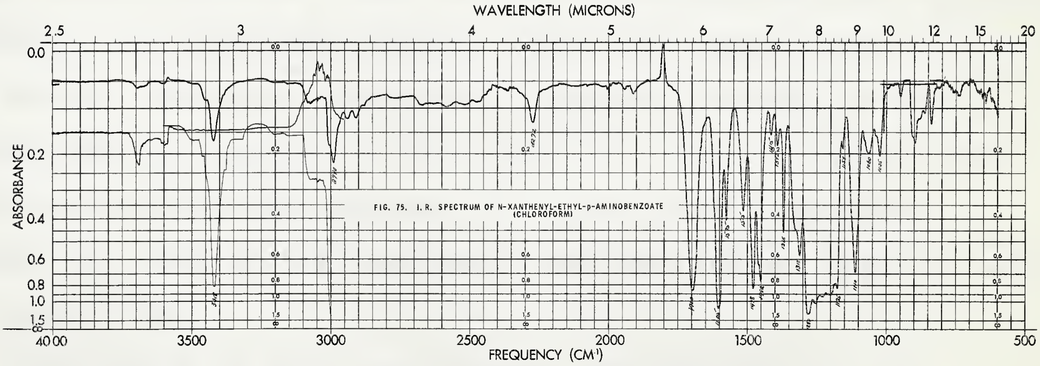
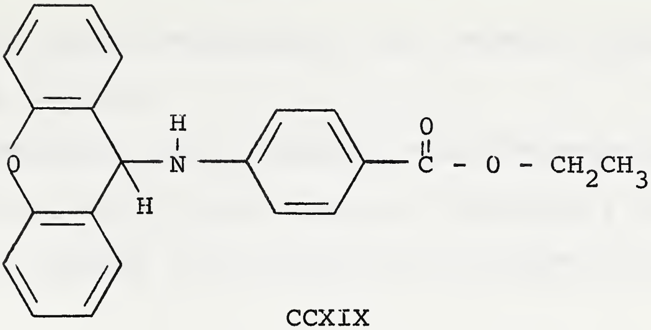


FIG. 75. I. R. SPECTRUM OF N-XANTHENYL-ETHYL-p-AMINOBENZOATE (CHLOROFORM)

shown to be substituted on the p-amino group (CCXIX). As predicted,



CCXIX

it too decomposed readily upon attempted recrystallization in the normal manner and thus it was also purified by crystallization from a mixed solvent at room temperature. Proof for the above structure CCXIX was again obtained from a comparison of the infrared spectrum of the parent compound (Figure 73) with that of the derivative (Figure 74). The former compound displayed the frequencies characteristic of a primary amino group at 3413, 3311 and 1639 cm^{-1}. The derivative, on the other hand, showed only a single band in the former region (3350 cm^{-1}) and, the NH deformation band had disappeared, diagnostic of a secondary amine. Since this compound was found to be slightly soluble in chloroform, a property uncommon to most of the derivatives prepared in this study, the infrared spectrum was also determined in this solvent. The spectrum in chloroform (Figure 75) verified the information derived from the spectrum taken as a nujol mull.

The results of this study suggest the applicability of xanthydrol as a reagent for a wide variety of ring-substituted primary aromatic amines. However, the instability associated with the C-N bond in these derivatives may also be predicted.

SUMMARY

The major points embodied in this research project may be presented as follows:

(a) Xanthydrol when condensed with N^1-monosubstituted sulfanilamides yielded either mono or dixanthenyl derivatives regardless of whether a deficiency or an excess of xanthydrol was employed.

(b) The site of substitution of the 9-xanthenyl radical, common to all sulfanilamides having a free para amino group, was shown to be the N^4-position in the sulfanilamide molecule.

(c) The C-N bond formed in the above substitution was found to be of a relatively weak nature rendering these derivatives unstable toward elevated temperatures, peroxides and perhaps other factors not immediately apparent.

(d) The site of substitution of the second 9-xanthenyl moiety, in the dixanthenyl derivatives, was shown to be one of the following three positions:

- (i) on the annular nitrogen atom of the heterocyclic ring, as a result of condensation with the imino tautomeric form of the sulfanilamide.
- (ii) on the extra-ring nitrogen atom at the N^1-position, reaction having occurred with the amino tautomeric form.
- (iii) on the activated 5-position of the pyrimidine ring.

(e) The C-N bond formed in the union of the 9-xanthenyl radical with the annular nitrogen atom was found to be much stronger in nature than that bonding the N^4-xanthenyl derivatives.

(f) Reactions of xanthydrol with sulfanilamides having pKa's in the vicinity of 5.5 were found to be autocatalytic in ethanol.

(g) The exceptional ease of formation of the xanthylium ion was postulated to be associated with the resulting stability of this carbonium ion by virtue of its aromatic character.

(h) In view of the instability of these derivatives, coupled with the fact that a considerable overlap exists in their melting points, the utility of xanthydrol as a reagent for the characterization of sulfanilamides is of limited value.

EXPERIMENTAL

All melting points recorded in this investigation were taken on a Thomas-Hoover capillary melting point apparatus, and are uncorrected.

Infrared spectra were recorded on Perkin-Elmer infrared spectrometers, Models 21 and 221 G. Ultraviolet spectra were obtained on a Beckman Model DK-2 spectrometer. Nuclear magnetic resonance spectra were recorded on a Varian Associates Model A-60 spectrometer.

Elemental analyses were performed by Messrs. Weiler and Strauss and by the Australian Microanalytical Service.

1. Experiments Related to the Preparation of 9-Xanthenyl Derivatives of Sulfanilamides.

Xanthydrol was obtained as the commercial product from Eastman Kodak Co. Inc., New York.

The Sulfanilamides were either obtained commercially or were generous gifts of the manufacturers.

Experiments Leading to the Adoption of a Generally Applicable Procedure.

A. To a solution of 0.5 g. (0.0025 mole) of xanthydrol in 10 ml. of glacial acetic acid was added an equimolar portion of the sulfanilamide, in the dry state, according to the method of Phillips and Frank (1). With all of the sulfanilamides, except sulfaphenazole, the reaction was almost immediate. In several instances the solution appeared to go clear before the derivative separated; however, with most sulfanilamides this was not apparent.

As a result, many of the products contained varying amounts of the unreacted sulfonamide. Sulfaphenazole, on the other hand, yielded a clear solution which after standing overnight afforded the product as light brown rosette-shaped crystals (m.p. 226-227°).

B. Sulfadiazine (1.26 g. (0.005 mole)) was dissolved in a minimum amount (700 ml.) of glacial acetic acid. To this solution was added 1 g. (0.005 mole) of xanthydrol dissolved in a mixture of 1.5 ml. of glacial acetic acid and 1.5 ml. of ethanol, according to the procedure described by Sawicki and Oliverio (5). After 3 weeks at room temperature a white product began to separate, complete separation not being apparent for approximately another week.

C. To a solution of 0.5 g. (0.0025 mole) of xanthydrol in 5 ml. of glacial acetic acid was added a solution of 0.54 g. (0.0025 mole) of sulfacetamide in 5 ml. of dioxane. After several minutes, a white product melting at 204-205° had separated. Other sulfanilamides tested proved to be too insoluble in dioxane.

D. Xanthydrol (0.5 g. (0.0025 mole)) and sulfacetamide (0.54 g. (0.0025 mole)) were dissolved in 25 ml. of 95% ethanol, the solution filtered, and 5 ml. of glacial acetic acid added. A product began separating after about 90 minutes at room temperature. The mixture was allowed to stand overnight, then filtered, washed with cold ethanol and dried. The resulting product melted at 202-203°, comparable with the derivative isolated in C. above. Once again, the other sulfanilamides were found to be too insoluble in ethanol to be adaptable to this procedure.

E. To a solution of 0.68 g. (0.0025 mole) of sulfamethizole in a minimum volume of acetone was added 0.5 g. (0.0025 mole) of xanthydrol in 10 ml. of glacial acetic acid. A product began

separating almost immediately. The crude product melted at 212-213° and depressed the melting point of the parent compound (m.p. 208°) to 170-195°. The identical procedure with sulfaethylthiadiazole yielded two distinct products. No attempt was made at isolating these products.

F. Equimolar amounts (0.0025 mole) of xanthydrol and sulfaethylthiadiazole were dissolved in a minimum of acetone, and 1 ml. of glacial acetic acid was added to the resulting solution. The mixture was stirred electromagnetically until the product separated (approximately 2 hours). Sulfamethoxypyridazine and sulfachloropyridazine also afforded products by this method. Most other sulfanilamides were not sufficiently soluble in acetone.

General Procedure for the Preparation of 9-Xanthenyl Derivatives.

To 1 g. (0.005 mole) of xanthydrol in 15 ml. of glacial acetic acid was added an equimolar amount of the sulfanilamide dissolved in a minimum volume (2-5 ml.) of N,N-dimethylformamide (DMF) while stirring electromagnetically. Stirring was continued for a period of 15 to 30 minutes beyond the time that the derivative first began separating. The product was collected on a medium porosity sintered glass funnel, washed well with cold ethanol and dried in a vacuum desiccator over P_2O_5.

2. Experiments Related to the Purification of the Crude 9-Xanthenyl Derivatives.

The Solvents were of ACS reagent grade quality, redistilled prior to their use.

Dioxane was rendered peroxide-free by a modification of the method of Dasler and Bauer (164). To 1 kg. of dioxane in a large container was added approximately 200 g. of alumina and the mixture

agitated continuously for 12 hours by means of a mechanical shaker. The alumina was then allowed to settle and the dioxane was passed through a column of fresh alumina. Complete removal was tested for by means of a potassium iodide in chloroform test solution (127).

Attempted Recrystallizations (Several Typical Examples).

(a) Xanthenyl Derivative of Sulfacetamide. The crude product, white in color, melted at 191-193°. Crystallization from 70% ethanol yielded a yellow product melting at 199-200°. A second recrystallization from this solvent lowered the melting point to 195-202°.

Dioxane-water (3:1) afforded a yellow product melting at 198-199°. A repeat of this procedure lowered the melting point to 194-196°.

Recrystallization from peroxide-free dioxane-water at room temperature gave a white product melting at 198.5-199°. A second crystallization raised the melting point to 201.5-202°. A third attempt, however, yielded a yellow product once again.

(b) Xanthenyl Derivative of Sulfaethylthiadiazole. The white crude product melted at 198-200°. Recrystallization from benzene gave a yellow product melting over a wide temperature range (180-205°). From methyl ethyl ketone the melting point was raised to 204-209° but the yellow color persisted. Chloroform-*n*-hexane lowered the melting point to 178-180°. Dioxane-*n*-hexane, on the other hand, afforded a mixture of white and yellow crystals.

(c) Xanthenyl Derivative of Sulfisoxazole. Recrystallization of the crude white product (m.p. 164-165°) from acetone-water yielded a yellow product melting at 165-166°. Dioxane-*n*-hexane also afforded a yellow product which melted at 175-177°.

Crystallization from peroxide-free dioxane-water at room temperature afforded a pale yellow product melting at 171-172°.

Attempted Crystallization in an Inert Atmosphere. The xanthenyl derivative of sulfapyridine was recrystallized from peroxide-free dioxane in an inert atmosphere employing an apparatus designed after Ulich (128). This device permitted the processes-dissolution of the substance, filtering of insoluble impurities, crystallization, collecting on a filter plate and washing - to be conducted in the one apparatus with complete exclusion of air. Dry nitrogen gas was employed as the inert gas. The first crystallization by this means yielded a white product, however, when the process was repeated a yellow product was obtained once again. Similar results were obtained with the derivatives of sulfamerazine, sulfamethizole and sulfaethylthiadiazole.

General Procedure for the Recrystallization of the Crude 9-Xanthenyl Derivatives. The crude derivative was dissolved in a minimum volume of pyridine (or other applicable solvent), at room temperature, and the resulting solution was filtered. Water (or *n*-hexane) was then added in small portions until the first sign of cloudiness or crystal formation was evident, and the solution set aside to allow for slow crystallization. After crystallization was complete, the product was collected on a sintered glass funnel (medium porosity) and washed several times with an appropriate mixture of the cold mixed solvents. The crystals were then dried in a vacuum desiccator over P_2O_5 and the melting point recorded. The procedure was then repeated until a constant melting point had been achieved. The recrystallizing solvents and melting points

for each of the xanthenyl derivatives are presented in Tables I and II which appear in the results and discussion section.

Attempted Removal of the Last Traces of Pyridine from the Recrystallized Samples.

(a) Drying at Elevated Temperatures. Drying of the analytical samples in a drying pistol at 75° (ethanol) resulted in a drop in the melting points accompanied by a color change from white to yellow in some instances. Thus, a drop of 3° was observed for the xanthenyl derivative of sulfamerazine with no apparent color change. The derivative of sulfamethiazine, on the other hand, turned yellow after 24 hours at 75° and the melting point now exhibited a very wide range (143-176°). The white recrystallized derivative of sulfaproxyline (m.p. 192-193°) became yellow after 18 hours at 75° and the melting point dropped to 187-189°. Drying at 55° (acetone) lowered the melting point of the xanthenyl derivative of sulfacetamide by 2°. Subjecting the xanthenyl derivative of sulfaethylthiadiazole to vacuum drying, at room temperature, for a period of 5 days failed to remove all of the pyridine from the sample.

(b) Drying in High-Velocity Dry Inert Gas. Two medium porosity sintered glass funnels were fixed with ground glass joints provided with glass hooks (129). The sample was placed in the filter having the outer joint and the apparatus was closed and secured with metal springs over the glass hooks. Dry nitrogen was passed upward through the sample for periods of approximately 10 minutes. After this time the device was opened and the sample examined for the odor of pyridine. As many as six 10-minute drying periods failed to remove the last traces of pyridine.

Removal of Pyridine from the Recrystallized Samples. Those derivatives which were recrystallized from pyridine-water or pyridine-hexane mixtures were accorded the following treatment: to the recrystallized sample contained in the sintered glass funnel, 20 ml. of n-pentanewere added and by means of a glass rod the sample was carefully reduced in particle size and mixed well with the solvent. The funnel was covered with a watch glass and the n-pentane allowed to drain from the sample under normal pressure. When the solvent had almost completely drained, vacuum was applied for several minutes. The process was repeated until the odor of pyridine was no longer discernible. Following this, the sample was dried in a drying pistol, at room temperature, for a period of 48 hours. Those derivatives recrystallized from acetone or chloroform mixtures were only subjected to the latter treatment. The dry purified samples were then submitted for carbon, hydrogen and nitrogen analyses. The results of these analyses are given in Tables I and II.

Attempted Isolation of the Yellow Decomposition Product by Thin Layer Chromatography. Glass plates, 20 x20 centimeters, were coated with silica gel G by means of a Desaga SII adjustable applicator to give a uniform 0.25 mm. layer of adsorbent (165). The plate was spotted with acetone solutions of xanthydrol, sulfacetamide, monoxanthenylsulfacetamide (crude) and the yellow product obtained after two recrystallizations of the latter from 70% ethanol. The plate was developed in a solvent system composed of chloroform-ethanol-heptane (1:1:1), a system employed successfully by Klein and Kho (166) for the separation of sulfonamides. The chromatogram was dried and sprayed with ceric sulfate 1% in

10% sulfuric acid (167). Three spots appeared for both the crude and recrystallized product corresponding to the derivative, sulfacetamide and xanthydrol. An unique spot that could be attributed to the decomposition product was not evident.

3. Experiments Related to the Structural Elucidation of the Mono- and Dixanthenyl Derivatives.

Nonaqueous Titration of the Derivatives as Acids. The titrations were carried out employing a modification of the method of Vespe and Fritz (130) which has been reported previously (168). The results for the monoxanthenyl derivatives are reported in Table III. The dixanthenyl derivatives, with the exception of that of sulfadimethoxine, did not titrate as acids. The latter afforded a recovery of 98.6%, corresponding to a molecular weight of 680.5 (Calc. 670.7).

N-Xanthenyl-*p*-Aminobenzoic Acid was prepared by condensing xanthydrol with *p*-aminobenzoic acid according to the general procedure given earlier. Four recrystallizations from acetone-*n*-hexane, at room temperature, yielded a white product melting at 173.5-174°.

Calc. for $C_{20}H_{15}NO_3$: C, 75.69; H, 4.77; N, 4.41.
Found : C, 75.80; H, 5.88; N, 4.27.

The compound could not be purified further.

Attempted Condensation of Benzoic Acid with Xanthydrol. Treatment of benzoic acid with xanthydrol according to the general procedure did not yield a product after 1 month at room temperature.

Attempted Condensation of *p*-Acetamido-Benzoic Acid with Xanthydrol. Condensation of the above reactants, employing the general procedure, afforded a small amount of a white product which was shown to be the starting acid. The material exhibited the same

melting point as p-acetamidobenzoic acid and a mixed melting point with the latter showed no depression.

3,5-Dibromo-4-Amino-Benzoic Acid was prepared by an adaptation of a bromination method described for phenols (169). To 1 g. of p-aminobenzoic acid in a minimum of ethanol, brominating solution (15 g. of KBr in 100 ml. of water plus 10 g. of bromine) was added dropwise with constant stirring. The yellow color continued to disappear until finally a white product separated. Addition of the brominating solution was continued until no more product precipitated. A 50 ml. portion of water was added, the product filtered and washed with a dilute solution of sodium bisulfite, followed by water. One recrystallization from ethanol yielded a white product which did not melt below 300°. Lit. unmelted at 280° (170).

Attempted Reaction of 3,5-Dibromo-4-Amino-Benzoic Acid with Xanthydrol. Condensation of the above reactants, according to the general procedure, afforded only the starting acid as evidenced from its identical infrared spectrum (see Figures 1 and 2).

p-Dimethylamino-Benzoic Acid was prepared by a modification of the method described by Décombe (171). Silver oxide was prepared by adding a solution of 15 g. (0.089 mole) of silver nitrate in 30 ml. of water to a solution of 7 g. (0.175 mole) of sodium hydroxide in 30 ml. of water according to the procedure of Campaigne and LeSuer (172). This resulted in a very thick mixture which could not be stirred properly and hence a further 90 ml. portion of water was added to permit adequate agitation. To this mixture were added 6.3 g. (0.0425 mole) of p-dimethylamino-benzaldehyde in small portions with continuous stirring. After addition was complete,

the mixture was stirred for a further 30 minutes. The black silver suspension was removed by suction filtration and washed with four 25-ml. portions of hot water. The cold combined filtrate and washings were acidified with glacial acetic acid precipitating 5.94 g. (85%) of p-dimethylamino-benzoic acid. After one recrystallization from 95% ethanol the acid melted at 233-236°. Lit. M.p. 235-238° (171).

Reaction of p-Dimethylamino-Benzoic Acid with Xanthydrol.

To 1 g. (0.005 mole) of xanthydrol in 15 ml. of glacial acetic acid was added 0.83 g. (0.05 mole) of p-dimethylaminobenzoic acid in 4 ml. of N,N-dimethylformamide. Stirring was continued for 18 hours after which time a white product had separated. The mixture was allowed to sit for a further 24 hours, filtered and washed well with cold ethanol. Further yields were recovered from the mother liquor affording a combined yield of 0.6 g. (35%). Six recrystallizations from ethanol yielded a white product melting at 155-156°.

Calc. for $C_{22}H_{19}NO_3$: C, 76.50; H, 5.54; N, 4.06

Found : C, 83.96 H, 6.29; N, 4.70

The resulting product was insoluble in sodium bicarbonate solution suggesting that it was no longer an acid. The infrared spectrum revealed no carbonyl absorption (Figure 4).

Recal. for $C_{21}H_{19}NO$: C, 83.69; H, 6.35; N, 4.65.

Found : C, 83.96; H, 6.29; N, 4.70.

p-(9-Xanthenyl)-N,N-Dimethylaniline was prepared according to the method described by Adriani (3). Recrystallization from ethanol afforded the title compound melting at 156-157.5°. Lit. m.p. 157-158° (53). This compound did not depress the melting point of the product isolated from the preceding reaction and exhibited an identical infrared spectrum (Figure 3).

Attempted Condensation of p-Dimethylamino-Benzaldehyde With Xanthydrol. Treatment of the above reactants, by the general procedure, did not afford a product after 1 month at room temperature.

N-Acetyl-p-Toluene-Sulfonamide was prepared according to the method of Kemp and Stephen (173). p-Toluene-sulfonamide (5.13 g. (0.03 mole)) was mixed with 3.06 g. (0.03 mole) of acetic anhydride in an evaporating dish and 3 drops of concentrated sulfuric acid were added. The mixture was warmed gently to give a clear solution. Upon cooling, the mixture solidified. A small amount of water was added and the mixture heated to boiling. The residue, after crystallization from water, melted at 140-141.5°. Lit. m.p. 137° (ethanol)(173). A mixed melting point with p-toluene-sulfonamide (m.p. 138-140°) showed a large depression.

Attempted Condensation of N-Acetyl-p-Toluene-Sulfonamide with Xanthydrol. Condensation of the above reactants, by the general procedure, failed to yield a product. Heating the reaction mixture at 80° for 40 minutes also failed to initiate a reaction.

N^1-Acetyl-3,5-Dibromosulfanilamide was prepared by the bromination of sulfacetamide employing the method, described previously, for preparing 3,5-dibromo-4-amino-benzoic acid. One recrystallization from 60% ethanol yielded white rosette-shaped

crystals melting at 191.5-192.5°. Lit. m.p. 193.8-194.6 (174). Molecular weight: Calc., 372.1; Found, 373.6 (Nonaqueous Titration).

Attempted Reaction of N^1-Acetyl-3,5-Dibromosulfanilamide with Xanthydrol. Condensation of the above reactants, employing the general procedure, failed to yield a product.

Color Tests for the Detection of Primary Aromatic Amines.

(a) Diazotization and Coupling. The quantitative procedure of Bratton and Marshall (136) was modified and applied in a qualitative capacity. To approximately 0.5 mg. of the xanthenyl derivative in several drops of N,N-dimethylformamide (DMF)were added 5 ml. of 0.5 N HCl followed by 5 ml. of 0.1% sodium nitrite. If a precipitate formed after the addition of the hydrochloric acid, sufficient DMF was added to redissolve the precipitate. After 3 minutes, 5 ml. of 0.5% ammonium sulfamate followed by 5 ml. of 0.1% N-(1-naphthyl) ethylene diamine di HClwere added to the clear solution. An immediate violet color was produced with each of the monoxanthenyl derivatives. A blank run treated in exactly the same manner remained colorless upon the addition of the coupling reagent.

(b) Furfural. The color test with furfural was performed as described by Hucknall and Turfitt (137). To 0.1 to 1 mg. of the derivative contained in a porcelain spot plate was added 1 drop of a 2% solution of furfural in glacial acetic acid. The liquid was allowed to evaporate spontaneously and the color produced was noted and compared with that from the parent compound. The parent compounds each afforded a violet color. The monoxanthenyl derivatives, on the other hand, remained colorless, except for the derivative of sulfaphenazole, which showed a faint violet coloration.

(c) Sodium Pentacyanoaquoferriate. This test was carried out by a modification of the method described by Anger (138). A small amount of the derivative was placed on a spot plate, and a few drops of acetone were added to dissolve the compound. One drop of the reagent solution, prepared by irradiating a 1% solution of sodium nitroprusside for 15 minutes under U.V. light, was added and the color produced was compared with that yielded by the parent compound. The parent sulfanilamides yielded a mauve or green color, whereas the xanthenyl derivatives gave an orange color. The derivative of sulfaphenazole afforded a pale brown coloration.

Effect of 0.5 N HCl on Monoxanthenyl-Sulfacetamide. To 0.5 mg. of the above derivative in a few drops of DMF were added 5 ml. of 0.5 N HCl as described under (a) above. Evaporation of the solvent yielded a residue which gave a positive test with furfural. A similar result was obtained when the solution was first neutralized with dilute sodium hydroxide and then evaporated to dryness.

Dixanthenyl-Sulfaguanidine was isolated, in a very low yield, when the reaction product from the condensation of sulfaguanidine and xanthydrol was recrystallized from pyridine-water at room temperature. The product melted at 230.5-231.5°.

Calc. for $C_{33}H_{26}N_4O_4S$: C, 68.97; H, 4.56; N, 9.75.

Found : C, 68.01; H, 4.64; N, 9.66.

N^1-(2,6-Dimethoxy-5-Bromo-4-Pyrimidyl)-3,5-Dibromosulfanilamide was prepared by the bromination of sulfadimethoxine according to the brominating procedure described earlier, using acetone rather than ethanol as the solvent. One recrystallization from 95% ethanol yielded a product melting at 190-191°. Molecular

weight: Calc., 547.1; Found, 548.6 (Nonaqueous Titration).

Attempted Condensation of N^1-(2,6-Dimethoxy-5-Bromo-4-Pyrimidyl)-3,5-Dibromosulfanilamide with Xanthydrol. Treatment of the above reactants, by the general procedure, afforded a product having an identical melting point to that of the tribromo compound. A mixed melting point showed no depression.

Reaction of Isobuzole with Xanthydrol, following the general procedure, yielded 2.15 g. (84%) of a white product. Recrystallization from 95% ethanol afforded white crystals melting at 170-170.5°. Calculation for a monoxanthenyl derivative gave the following results:

Calc. for $C_{26}H_{25}N_3O_4S_2$: C, 61.52; H, 4.96; N, 8.28.
Found : C, 61.37; H, 4.95; N, 8.17.

N^1-(5-Ethyl-1,3,4-Thiadiazol-2-yl) 3,5-Dibromosulfanilamide was prepared by the bromination of sulfaethylthiadiazole in acetone by the brominating procedure described earlier. Recrystallization from 70% ethanol afforded a white product melting at 146-147°, with previous softening. Lit. m.p. 197° (175).

Reaction of N^1-(5-Ethyl-1,3,4-Thiadiazol-2-yl)-3,5-Dibromosulfanilamide with Xanthydrol, employing the general procedure, afforded 1.20 g. (82%) of a white product. Three recrystallizations from 95% ethanol yielded a product melting at 200-201°. Calculation for a monoxanthenyl derivative provided the following results:

Calc. for $C_{23}H_{18}Br_2N_4O_3S_2$: C, 44.38; H, 2.92; Br, 25.68; N, 9.00
Found: C, 44.69; H, 3.18; Br, 25.42; N, 9.08

Condensation of Phthalylsulfathiazole with Xanthydrol, according to the general procedure, yielded 2.48 g. (84%) of a pale white product. Recrystallization from acetone afforded a product melting at 153-153.5°. Calculation for a monoxanthenyl derivative gave the following results:

Calc. for $C_{30}H_{21}N_3O_6S_2$: C, 61.73; H, 3.63; N, 7.20.
Found : C, 61.15; H, 3.90; N, 7.34.

Reaction of Succinylsulfathiazole with Xanthydrol. Condensation of the above reactants, following the general procedure yielded 2.04 g. (75%) of a product shown to be a monoxanthenyl derivative. Recrystallization from dioxane-water gave a white product melting at 173.5-174.5°.

Calc. for $C_{26}H_{21}N_3O_6S_2$: C, 58.31; H, 3.95; N, 7.85.
Found : C, 57.94; H, 4.07; N, 8.13.
Molecular Weight : Calc., 535.6;
Found, 545.5 (Nonaqueous Titration).

Reaction of p-Nitrosulfathiazole with Xanthydrol, following the general procedure similarly yielded a monosubstituted derivative. Recrystallization from chloroform-*n*-hexane afforded a yellow product melting at 166-166.5°.

Calc. for $C_{22}H_{15}N_3O_5S_2$: C, 56.76; H, 3.25; N, 9.03.
Found : C, 56.10; H, 3.30; N, 8.31.

The compound could not be purified further.

N^4-(9-Xanthenyl)-N^1-Acetyl-N^1-(6-Methoxy-3-Pyridazinyl)-Sulfanilamide was obtained by the condensation of N^1-acetyl-sulfamethoxypyridazine with xanthydrol. To 1 g. (0.005 mole) of xanthydrol in 15 ml. of glacial acetic acid was added 1.63 g. (0.005 mole) of N^1-acetyl sulfamethoxypyridazine in the solid

state. The mixture was stirred for 30 minutes, filtered and washed with cold ethanol. Three recrystallizations from pyridine-water, at room temperature, followed by one from acetone-hexane also at room temperature afforded a white product melting at 217.5-218.5°.

Calc. for $C_{26}H_{22}N_4O_5S$: C, 62.14; H, 4.41; N, 11.15.

Found : C, 60.81; H, 4.41; N, 11.20.

The derivative could not be purified further.

N^4-(9-Xanthenyl)-N^1-Acetyl-N^1-(3,4-Dimethyl-5-Isoxazolyl)-Sulfanilamide was obtained from the reaction of N^1-acetyl-sulfisoxazole with xanthydrol following the general procedure. Two recrystallizations from acetone-water followed by one from acetone-hexane, each at room temperature, yielded a white product melting at 188-189°.

Calc. for $C_{26}H_{23}N_3O_5S$: C, 63.79; H, 4.74; N, 8.58.

Found : C, 63.90; H, 4.74; N, 8.65.

4. Experiments Related to the Reactivity of Xanthydrol.

N^4-(9-Xanthenyl)-N^1-Acetyl-Sulfanilamide was obtained from the condensation of sulfacetamide with xanthydrol in 95% ethanol. Xanthydrol (1.84 g. (0.0093 mole)) and 2.0 g. (0.0093 mole) of sulfacetamide were dissolved in 40 ml. of 95% ethanol. The resulting solution was slightly turbid and hence was filtered. During the filtering process a white product began to separate in the filtrate. The product when collected weighed 1.75 g. (48%) and melted at 203.5-204.5. A mixed melting point with an authentic sample of the title compound showed no depression.

Dixanthenylsulfamethizole was obtained from the condensation of sulfamethizole with xanthydrol in 95% ethanol. To 1 g. (0.005 mole) of xanthydrol in 30 ml. of 95% ethanol was added 0.68 g. (0.0025 mole) of sulfamethizole in the dry state. Sufficient ethanol was added to yield a clear solution. Standing overnight afforded 0.91 g. (57%) of a white product melting at 198-200°. A mixed melting point with an authentic sample of the title compound (m.p. 206.5-207) yielded a melting point of 200-203°. A mixed melting point with the parent sulfanilamide (m.p. 208°), on the other hand, was depressed to 177-187°.

N-(9-Xanthenyl)-p-Amino-Benzoic Acid was obtained, in very low yield, from the reaction of p-aminobenzoic acid with xanthydrol in 95% ethanol. To 1 g. (0.0025 mole) of xanthydrol in 25 ml. of 95% ethanol was added 0.7 g. (0.005 mole) of p-aminobenzoic acid in 10 ml. of 95% ethanol. After two weeks at room temperature a small amount of a white product was recovered which, from its melting point and mixed melting point determinations proved to be the title compound.

Attempted Condensation of Sulfapyridine with Xanthydrol in Ethanol. Mixing of equimolar portions of sulfapyridine and xanthydrol, each in a minimum volume of ethanol, afforded no reaction. Only a small amount of the parent sulfanilamide was recovered.

N^4-(9-Xanthenyl)-N^1-Acetyl-Sulfanilamide was obtained from the following reaction: Equimolar portions (0.005 mole) of xanthydrol and sulfacetamide were dissolved in a minimum volume of acetone and the resulting solution filtered. After 1 month at room temperature, the solution remained clear. The solution was then treated with 25 ml. of 95% ethanol and again left undisturbed. After

several days a white crystalline product melting at 205-207° had separated. A mixed melting point with an authentic sample of the title compound showed no depression.

Dixanthenyl-Urea was obtained from the following reaction: To 1 g. (0.005 mole) of xanthydrol in 40 ml. of 95% ethanol was added 0.15 g. (0.0025 mole) of urea dissolved in a minimum volume of ethanol. One month at room temperature failed to yield a product. At this point, 10 ml. of glacial acetic acid were added to the mixture with continuous stirring. After 25 minutes a white product melting at 250-251° was obtained. Lit. m.p. 250-258° (69).

5. Experiments on the applications of this Reaction

N^4-(9-Xanthenyl-N^1-Phenyl-Sulfanilamide was prepared by condensing sulfanilanilide with xanthydrol according to the general procedure. Five recrystallizations from acetone-hexane, at room temperature yielded colorless needles melting at 185-186°.

Calc. for $C_{25}H_{20}N_2O_3S$: C, 70.07; H, 4.71; N, 6.54.

Found: : C, 69.65; H, 4.78; N, 6.56.

Molecular Weight: Calc., 428.5;
Found, 432.5 (Nonaqueous Titration).

N^3-Xanthenyl-N^1-(5-Chloro-2-Pyrimidyl)-Metanilamide was prepared by condensing metachloridine with xanthydrol following the general procedure. Three recrystallizations from pyridine-water followed by two from acetone-hexane, each at room temperature, afforded a white product melting at 221.5-222°.

Calc. for $C_{23}H_{17}ClN_4O_3S$: C, 59.41; H, 3.69; N, 12.05.

Found : C, 59.38; H, 3.70; N, 12.11.

Molecular Weight: Calc., 464.9;
Found, 464.7 (Nonaqueous Titration).

N-(9-Xanthenyl) Ethyl p-Amino Benzoate was prepared by the reaction of ethyl p-amino-benzoate with xanthydrol according to the general procedure. Three recrystallizations from acetone-water, at room temperature, yielded a white product melting at 200-201°.

Calc. for $C_{22}H_{19}NO_3$: C, 76.50; H, 5.54; N, 4.06.
Found : C, 76.52; H, 5.72; N, 4.15.

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